The role of parasites in host speciation

Testing for parasite-mediated divergent selection at different stages of speciation in cichlid fish

Tiziana P. Gobbin

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aquatic research

The research presented in this thesis was carried out

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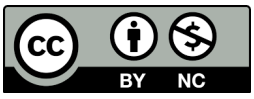

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The role of parasites in host speciation

Testing for parasite-mediated divergent selection at different stages of speciation in cichlid fish

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1

1

General introduction

Tiziana P Gobbin

1.1. SPECIATION

How do species arise? – Explaining the mechanism by which new species arise has been a central question in biology since the formulation of the theory of evolution by Darwin and Wallace (Wallace, 1855; Darwin, 1859). New species can be the result of non-selective forces or of two distinct selective forces: natural selection and sexual selection, the struggle to survive and reproduce. In population biology, speciation is often defined as the evolution of significant reproductive isolation between two or more previously interbreeding populations.

Mechanisms of speciation – Our understanding of what factors and events are playing a role in initiating, promoting, stabilizing and completing the emergence of new species is still incomplete. Speciation can act by three main alternative mechanisms: speciation not selection based (which is driven by chance events, e.g. polyploidization, genetic drift; Coyne & Orr, 2004), uniform-selection speciation (in which populations exposed to similar selection fix different genetically-based adaptations; Schluter, 2001; Nosil & Flaxman, 2010) and ecological speciation (which is the focus of this thesis). Ecological speciation occurs when populations experience ecologically based divergent selection (Rundle & Nosil, 2005; Seehausen et al., 2008; Schluter, 2009; Nosil, 2012) and adapt to it by genetic divergence in morphology, physiology and/or behaviour, that reduce reproductive compatibility (Mayr, 1963; Schluter, 2000b; Rundle & Nosil, 2005). Ecological divergent selection can affect reproductive isolation incidentally (natural selection for certain phenotype traits that affect the likelihood of mating) or directly (selection for mating signals or mating preferences).

1.2. PARASITE-MEDIATED SPECIATION

Parasite-mediated speciation – Ecological speciation can arise from adaptations and counter-adaptations between two biotic actors (e.g. parasite-host) (Schluter, 2001; Decaestecker et al., 2007). Parasites impose a fitness cost on hosts, that may adapt by evolving an immune response. An immune defence against parasites can be costly (Sheldon & Verhulst, 1996) and may be at the expenses of other physiological processes (e.g. carotenoids may be used in immune defence as well as in sexually selected colour signals; Folstad & Karter, 1992; Lozano, 1994; Hill, 1999; Baeta et al., 2008). Therefore, specialised resistance would only be favoured if its benefits outweigh the cost of reduced investment in those other processes (i.e. allocation trade-off). Host populations infected by different parasite numbers and/or species are assumed to be subjected to different selective pressures and to face different trade-offs, to which they are expected to adapt by evolving different immune strategies. Host individuals adapted to their specific parasite threat are favoured by natural selection and possibly more often chosen as mates by individuals facing similar parasite challenges, which may promote reproductive isolation between host

populations differing in infection. In the context of speciation, parasites are considered to be potential drivers of, or contributors to, ecological divergence (Buckling & Rainey, 2002; Summers et al., 2003; Karvonen & Seehausen, 2012); as investigated in the present thesis.

Prerequisites for parasite-mediated speciation – Parasite-mediated speciation can operate in host populations if three main prerequisites are satisfied (Rundle & Nosil, 2005; Karvonen & Seehausen, 2012): *i)* parasite infections differ within or between host populations, *ii)* the direction of parasite-mediated selection is consistent through time; *iii)* parasite infections impose a fitness cost on the host.

First, parasite infections should vary within or between host populations, in magnitude or in parasite community composition. Variation in infection depends on the host risk of infection (determined by host ecology, such as microhabitat and trophic specializations) and on the host immune response (resistance, tolerance).

The second prerequisite for parasite-mediated speciation is that the direction of divergent parasite-mediated selection remains consistent over time. Stochastic or frequency-dependent temporal fluctuations in parasite abundances could cause variation in the strength and direction of parasite-mediated selection and the extent to which selection is divergent. Anyway, divergence between host populations would not be hampered if the direction of divergent selection is consistent over time in the face of fluctuations in selection strength (i.e. host population A consistently has a higher infection of a given parasite species than host population B).

Third, parasitic infection should impose a cost on host fitness, thereby exerting selection for increased resistance or tolerance on the host. Parasites can negatively affect host fitness in several non-exclusive ways, such as decreasing food intake, growth, sexual attractiveness, competitive ability, immune response (Lehmann, 1993; Coop & Holmes, 1996; Sorensen & Minchella, 1998; Taskinen, 1998; Johnsen & Zuk, 1999; Barker et al., 2002; Bollache, 2015) and survival rates (Gulland et al., 1993).

Mechanisms of parasite-mediated speciation – Parasite-mediated divergent selection can promote the evolution of reproductive isolation, through three non-exclusive mechanisms (MacColl, 2009a; Karvonen & Seehausen, 2012): *i)* reduction of hybrid/immigrant fitness, *ii)* direct effects of the genes of the immune system on mate choice and *iii)* parasite-mediated sexual selection.

Hybrids might be disadvantaged (i.e. higher infection levels compared to parentals) because of a possible heterozygote disadvantage in immunity. Hybrids may have reduced survival and/or low mating success, resulting in a fitness disadvantage, which could contribute to reproductive

isolation between their parental populations. Higher parasite infection was previously observed in hybrids of sympatric species of *Daphnia* in a Swiss lake (Wolinska et al., 2004) and in hybrids of lake and river populations of sticklebacks in Germany (Eizaguirre et al., 2012a). Alternatively, heterozygosity at MHC loci may allow an immune response to a broader array of parasite peptides than is possible in more homozygous genotypes, which could result in lower infection in hybrids than in parentals (Moulia et al., 1995). This would favour hybrids and hence hamper the evolution or maintenance of reproductive isolation between host populations.

Immigrants may be disadvantaged if they do lack immunity against local parasites, but they may also be less receptive to specialized local parasites. Higher parasite infection in immigrants was observed in white-crowned sparrows immigrating from a nearby region differing in singing dialect (MacDougall-Shackleton et al., 2002) and in marine sticklebacks experimentally moved to lakes (MacColl & Chapman, 2010).

Reproductive isolation between host populations can also arise through immune-mediated mate choice or parasite-mediated selection on sexual signals. In vertebrates, mate choice can involve the major histocompatibility complex (MHC) (Milinski, 2006; Eizaguirre & Lenz, 2010), a large and highly polymorphic family of genes also involved in adaptive immunity against parasites (Blais et al., 2007; Eizaguirre et al., 2009a; Lenz et al., 2013). MHC genes may be subjected to divergent selection: if some alleles are more efficient against a specific parasite, they will be selected in environments where such parasite is important (Eizaguirre et al., 2009a), potentially leading to mate choice that would provide offspring with higher resistance as a byproduct (Nuismer et al., 2008; Eizaguirre & Lenz, 2010; Eizaguirre et al., 2010). On the other hand, since intermediate MHC diversity is optimal (Germain, 1994; Woelfing et al., 2009), host individuals may prefer partners with dissimilar MHC types, as observed in Atlantic salmon (Landry et al., 2001; Consuegra & Leaniz, 2008), stickleback (Milinski et al., 2005), Brown trout (Forsberg et al., 2007), Sand lizard (Olsson et al., 2003) and humans (Milinski, 2006). Sticklebacks have been extensively studied in this context, providing support for a driving role of MHC in parasite-mediated mate choice (Reusch et al., 2001; Aeschlimann et al., 2003). Females choose mates that optimize the number of MHC alleles in their offspring (Reusch et al., 2001; Aeschlimann et al., 2003; Milinski et al., 2005) and frequency of host MHC alleles shifts after only one generation under different parasite selection (Eizaguirre et al., 2012b).

Issues of studying parasite-mediated speciation – Direct evidence for parasite-mediated speciation is very limited, because of two main issues. First, it is difficult to interpret which interaction partner (parasite or host) is driving diversification of the other because most studies are correlational. Some studies showed that parasite speciation is triggered by host diversity (Krasnov et al., 2004; Nishimura et al., 2011), some that host speciation is driven by parasites (Price et al., 1986; Fincher & Thornhill, 2008) and others that parasites and hosts have co-speciated (Paterson & Poulin, 1999; Dabert et al., 2001). The second difficulty in investigating

parasite-mediated speciation is disentangling the diversifying effects of parasites from other (ecological) causes of host divergence, such as trophic or habitat differentiation (Knudsen et al., 2010). Hosts of different species or of conspecific populations with different ecological specializations may harbour different parasite communities, but this does not imply parasite-mediated speciation as differences in infection may simply accumulate as a consequence of the speciation process, rather than driving it. To address this, it is necessary to study host populations at early stages of speciation and/or host groups varying in the extent of genetic differentiation.

Support for parasite-mediated speciation – Most evidence supporting parasite-mediated speciation comes in piecemeal, with different studies supporting some specific aspects but few if any demonstrating the complete chain of evidence.

***i)* Parasite-induced fitness cost**. Parasites need to impose a fitness cost in order to exert divergent selection on hosts. This has been reported in a wide range of taxa (e.g. mammals, Careau et al., 2010; fish, Milinski & Bakker, 1990; crustaceans, Stirnadel & Ebert, 1997; Tellenbach et al., 2007; angiosperms, Segar et al., 2018; birds, Hamilton & Zuk, 1982). The fitness cost imposed by the same parasite may also differ between host species/populations (as in two sympatric congeneric amphipods infected by a trematode, Thomas et al., 1995).

***ii)* Differences in infection between host species/populations**. In order to be subjected to parasite-mediated divergent selection, hosts need to differ in infection. Parasitic infections differ at several levels of host differentiation: between sympatric closely related host species (rodents in Senegal, Brouat et al., 2007; woodrats in California, Bechtel et al., 2015; bush babies in Gabon, Boundenga et al., 2018), between allopatric conspecific host populations (high/low elevation Mediterranean lizards, Carbayo et al., 2018; temperate/tropical fruitfly, Tinsley et al., 2006; Lake Tanganyika cichlids, Raeymaekers et al., 2013; Grégoir et al., 2015; Hablützel et al., 2016; perch in Finland, Karvonen et al., 2005), between sympatric host species (amphipods of French rivers, Galipaud et al., 2017; benthic/limnetic lake sticklebacks, MacColl, 2009a; Lake Tanganyika cichlids, Vanhove et al., 2015; Kmentová et al., 2016; Hablützel et al., 2017; Hayward et al., 2017), between sympatric morphs of the same species (in Arctic charr, Dorucu et al., 1995; Knudsen et al., 1997; Knudsen et al., 2003).

***iii)* Temporal consistency of parasite-mediated selection**. The direction of infection differences need to be consistent through time in order to maintain the direction of divergent selection. Temporally consistent infection differences have been observed in cichlids of Lake Tanganyika (Raeymaekers et al., 2013), in icefish from the Antarctic Sea (Mattiucci et al., 2015) and in lake sticklebacks from Scotland (De Roij & MacColl, 2012).

***iv)* Differences in infection coincide with differences in immunity**. Variation in parasite-mediated selection among host populations is expected to lead to different adaptations in immunity in those populations. Immune response is adapted to the local parasite challenge in stickleback lake-river ecotypes (Scharsack et al., 2007) and in fruit fly populations (Corby-Harris & Promislow, 2008). Other studies have found that the diversity of MHC alleles varies with the infection load (in water python, Madsen & Ujvari, 2006; in stickleback, Wegner et al., 2003) and with the parasite community composition (Lake Malawi cichlids, Blais et al., 2007). Immunogenetic differentiation increased with infection levels of intestinal parasites in cichlids of Lake Tanganyika (Meyer et al., 2019).

***v)* Link between infection/immunity and mate choice**. Host divergence in infection and/or in immunity may influence mate choice patterns, potentially contributing to reproductive isolation. In several taxa, females have been observed to prefer males harbouring fewer parasites, often associated with variation in sexual signals, in fish (stickleback, Milinski & Bakker, 1990; cichlids, Maan et al., 2008) and birds (pheasant, Hillgarth, 1990; red jungle fowl, Zuk et al., 1990; barn swallow, Moller, 1990).

To summarize, parasites can impose a temporally consistent selection by reducing host fitness and can induce an immune response, which may diverge in host populations facing different parasite threats. Immune response based on MHC also affects mate choice, which may ultimately lead to reproductive isolation. However, there is no report of a case with a complete evidence chain. It is still unclear how common and how important parasite-mediated speciation is, under which circumstances it can happen and at what stage of the speciation process.

Research questions – In this thesis I investigate whether parasites drive or contribute to host speciation. To this end, I asked the following questions. Do sympatric and closely related host species differ in infection patterns? Is the direction of parasite divergent selection consistent over time? Does differentiation in infection precede (neutral) genetic differentiation? To address these questions, I study the haplochromine cichlids of Lake Victoria and their macroparasites. I first explain why I choose this study system and then I will introduce it.

1.3. STUDY SYSTEM

Why study parasite-mediated selection in cichlid fish – A previous study in Lake Tanganyika found that *Cichlidogyrus* flatworms speciated synchronically with tropheine cichlids (Vanhove et al., 2015), providing some indication for the possibility of parasite-mediated speciation in cichlids. This was supported by a congruence between host and parasite phylogenetic trees, by molecular clock analysis, and by the rarity of host switching (despite ample opportunities for it). However, it is still unclear if parasites drove host speciation or vice-versa.

Several observations suggest that parasite-mediated speciation may occur in cichlids. First, cichlids are a species-rich lineage that are characterised by spatially fragmented populations as well as strong ecological niche differentiation. These features render them generally prone to diverge under local co-evolutionary dynamics (Thompson, 2005). In addition, their diversity in ecological niches suggests that different species may be exposed to different parasites (as previously reported in Lake Tanganyika, Hablützel et al., 2017; Hayward et al., 2017, and in Lake Victoria, Maan et al., 2008; Karvonen et al., 2018). Second, cichlid population densities can be high, favouring the spread of infectious diseases (Ribbink et al., 1983; Fenton et al., 2002). This is supported by the positive association between both host density and abundance and diversity of parasites (Hayward et al., 2017). Third, parasitism has been shown to affect the mating of cichlid species (Taylor et al., 1998; Maan et al., 2006b), which could provide a mechanism by which parasite-mediated selection contributes to reproductive isolation. Fourth, MHC genes are rapidly evolving in cichlids (Blais et al., 2007), suggesting rapid adaptation to different parasite pressures between lineages. Finally, the African Great Lakes are relatively stable environments, without seasonal breaks or diapause in parasite life cycles, indicating that the direction of parasite-mediated selection can be fairly consistent over time.

1.3.1. Hosts: African cichlid fish

Cichlids – Cichlid fish (Telostei: Perciformes: Cichlidae) include more than 2'000 species distributed across Central and South America, Africa the Middle East, Madagascar, southern India and Sri Lanka (Kocher, 2004). Cichlids speciated in many African lakes, including the Great Lakes Tanganyika, Malawi and Victoria (Fryer & Iles, 1972; Kocher, 2004; Seehausen, 2006). There, they display exceptionally high species richness, large diversity in morphology, ecology and behaviour, and high levels of endemism (Fryer & Iles, 1972; Turner et al., 2001; Kocher, 2004; Wagner et al., 2012a; Salzburger et al., 2014; Wagner et al., 2014). The species flocks that rapidly evolved in the African Great Lakes represent some of the most extensively studied examples of adaptive radiation (Fryer & Iles, 1972; Greenwood, 1974; Kornfield & Smith, 2000; Kocher, 2004; Won et al., 2005; Seehausen, 2006; Wagner et al., 2013; McGee et al., 2020).

Lake Victoria cichlids – The speciation rate of Lake Victoria cichlids is faster than that in any other known fish radiations, as shown by the phylogeny of >1'700 cichlid species (McGee et al., 2020). Two distantly related lineages hybridized in the Lake Victoria region about 100'000 years ago, providing the genetic variation for subsequent adaptive radiations of the Victoria region lakes (Seehausen et al., 2003; Meier et al., 2017a). Until 14'600 years ago Lake Victoria was completely dry (Johnson et al., 1996; Stager & Johnson, 2008). After its refilling, the lake was colonized by at least four cichlid lineages (Seehausen et al., 2003; Meier et al., 2017a). This hybrid swarm provided the genetic variation that, together with ample ecological opportunity, allowed rapid adaptive radiation (Seehausen, 2004; Salzburger, 2018). Thus, the Lake Victoria cichlid flock (approximately 500 known species) evolved *in situ* over that short period of time (Johnson et al.,

2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a). Despite the recent origin of the lake, the Victorian cichlids are ecologically similarly diverse as the older Malawi and Tanganyika cichlid radiations (Young et al., 2009). Because of young age and wide range of ecological specializations, cichlids of Lake Victoria constitute an interesting system to study the early stages of adaptive radiation.

Haplochromines – Most cichlids inhabiting Lake Victoria belong to the tribe of *haplochromini*. They display a wide range of shapes and colours, as well as ecological differentiation and trophic specializations (Fryer & Iles, 1972; Witte & van Oijen, 1990; Seehausen, 1996b; Bouton et al., 1997). Species assemblages of haplochromines can be very rich, with up to 35 species occurring in sympatry on single rocky islands (Seehausen, 1996b). Sexual dimorphism is widespread: males often express conspicuous coloration, while females tend to have a cryptic greyish coloration (Seehausen & van Alphen, 1999; Maan et al., 2004; Kidd et al., 2006). Females often show behavioural mating preferences for males of their own species, using male coloration as choice criterion (Seehausen & van Alphen, 1998; Maan & Sefc, 2013; Selz et al., 2014). Since colourful males tend to be less infected (Maan et al., 2008) and mating with parasite-resistant males provides good genes to the offspring (Hamilton & Zuk, 1982), female choice may be under parasite-mediated selection. This in turn could possibly strengthen reproductive isolation in host populations differing in infection profiles.

To radiate or not to radiate – Beside radiations, there are also hundreds of cases in which cichlids colonized lakes but did not speciate (Seehausen, 2006; Wagner et al., 2012a; Wagner et al., 2013). In Lake Victoria, cichlid species that failed to speciate after colonizing the lake are: *Astatoreochromis alluaudi* (Pellegrin, 1904), *Pseudocrenilabrus multicolor* (Schöller, 1903), *Oreochromis variabilis* (Boulenger, 1906) and *Oreochromis esculentus* (Graham, 1928). These lineages are older than and distantly related to the ancestor of the Lake Victoria radiation, although some of them are very similar to the latter in most life history and reproductive traits. In this thesis, I will take advantage of the co-occurrence within Lake Victoria of haplochromine lineages that did not speciate and the members of the radiation in order to study the potential role of parasites in cichlid speciation.

Replicates of species pairs of *Pundamilia* – Part of my thesis focuses on replicate sympatric pairs of blue and red forms of *Pundamilia* (**Fig. 1.3**) that vary in their time since speciation and the associated extent of genetic differentiation. This allows me to assess at what stage of speciation infection differences arise. The blue *Pundamilia pundamilia* (Seehausen et al., 1998) and the red *Pundamilia nyererei* (Witte-Maas & Witte, 1985) inhabit the clear waters of the southeastern part of Lake Victoria and may be nearly as old as modern Lake Victoria, i.e. approximately 15'000 years and 7'500 generations. About 1'200 generations ago, *P. pundamilia* colonized the Mwanza Gulf (e.g. Kissenda, Python and Luanso Islands), followed more recently by *P. nyererei*. Admixture between these two species generated a hybrid population (Meier et al., 2017b; Meier et al.,

2018). In parts of its range (including Python and Kissenda Islands), this hybrid population later speciated into sympatric species pairs of blue and red *Pundamilia* that resemble the original species (referred to as *P.* sp. 'pundamilia-like' and *P.* sp. 'nyererei-like', respectively). At Luanso Island, with very murky waters, the population (*P*. sp. 'Luanso') is panmictic, but it varies in male colouration with blue, red and intermediate colour morphs. Except at Luanso, the blue and red forms differ in diet and have parapatric depth ranges: blues are benthic insectivores inhabiting crevices in shallow waters (mainly 0-4 m), while reds are insectivores/zooplanktivores and occur in deeper waters (mainly 4-10 m) (Maan et al., 2006a; Seehausen et al., 2008; Castillo Cajas et al., 2012). Divergence in depth occupation coincides with exposure to different visual environments (blues: full-spectrum light environment, reds: red-shifted light spectrum) and with differences in visual pigment allele frequencies and opsin gene expression (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019). Visual cues (i.e. colouration) are used by females of both forms to choose their males (Seehausen & van Alphen, 1998; Haesler & Seehausen, 2005; Stelkens et al., 2008; Selz et al., 2014). At clear water locations (e.g. Makobe), assortative mating is strong and there are no indications of recent geneflow. At more southern locations, with lesser water transparency, ecological differentiation and assortative mating are weaker and there is evidence for low levels of hybridization and gene flow (e.g. Kissenda, Python) (Seehausen et al., 2008; Meier et al., 2017b).

1.3.2. Parasites: macroparasites infecting cichlids

What is a parasite? – With more than half of all species of animals being parasites, parasitism is the commonest lifestyle on Earth (Poulin, 1996; Windsor, 1998). Parasites live at the expense of other organisms, called hosts, living on the outside (ectoparasites) or the inside (endoparasites) of the host body. The parasite life cycle can be direct (only one host species needed to complete the parasite development) or indirect (one or more intermediate host species are needed in different life stages of the parasite). The intermediate host is the one where immature parasites undergo ontogenetic developmental and morphological changes, and often acts as a vector for the parasite to reach its final host. The final host is the one where parasites reach the adult or sexually mature stage.

Parasites infecting fish – Fishes are intermediate or final hosts for a wide range of micro- and macroparasite taxa: protists, monogeneans, nematodes, trematodes, bivalve molluscs, crustacean copepods, acanthocephalans and leeches (Roberts, 2012). Fish are even parasitized by other fish and by cyclostomes. All monogeneans, most arthropods and some nematodes have a direct life cycle, in which fish may act as final and only hosts. Many nematodes and trematodes have a complex life cycle, in which fish are intermediate hosts and piscivorous birds are often the final hosts. Many fish parasites have a free-living stage, as larvae or eggs, that is released into the environment before actively or passively infecting a host.

Monogeneans – Flatworms (Plathyhelminthes: Monogenea) are mainly ectoparasites of fishes (but also of amphibians). They have a specialized attachment organ (haptor) that displays large morphological variation, which is used by taxonomists to distinguish species (Paperna, 1979; Pariselle & Euzet, 1994; Whittington & Chisholm, 2008). They can move along fish gills (Kearn, 1987), possibly driven by the need to find a mate (they are unable to self-fertilize despite being hermaphrodites) and/or to avoid competition. Eggs are released into the water column and ciliated larvae hatch after a few days (Bychowsky et al., 1957; Paperna, 1996). Larvae have a short free-swimming life span and must find and infect a suitable host within 4-6 hours (Prost, 1963; Pariselle et al., 2003) and at the first attempt, because they are unable to switch host after attachment (Paperna, 1996). West African cichlids are parasitized by five monogenean genera: the ectoparasites *Cichlidogyrus* (Paperna, 1996), *Gyrodactylus* (von Nordmann, 1832), *Scutogyrus* (Pariselle & Euzet, 1995b), *Onchobdella* (Paperna, 1968) and the endoparasites *Enterogyrus* (Paperna, 1963) and *Urogyrus* (Bilong-Bilong et al., 1994).

Cichlidogyrus (**Fig. 1.1a-f**) is the most diverse genus of monogeneans. It is a gill parasite that primarily infects cichlids (but it was also found in two other fish families; Pariselle & Euzet, 2009; Messu Mandeng et al., 2015), displaying high species-specificity (i.e. individual species infecting only one or few related cichlid species; Pariselle et al., 2015). Adults have a flattened elliptical body (0.3-0.4 mm) with a posterior haptor used to attach to gill secondary lamellae. Attachment may cause secretion of mucus, hyperplasia and neutrophils infiltration (Igeh & Avenant-Oldewage, 2020). They are hermaphrodites that cross-fertilize on the host. Larvae are free-living,

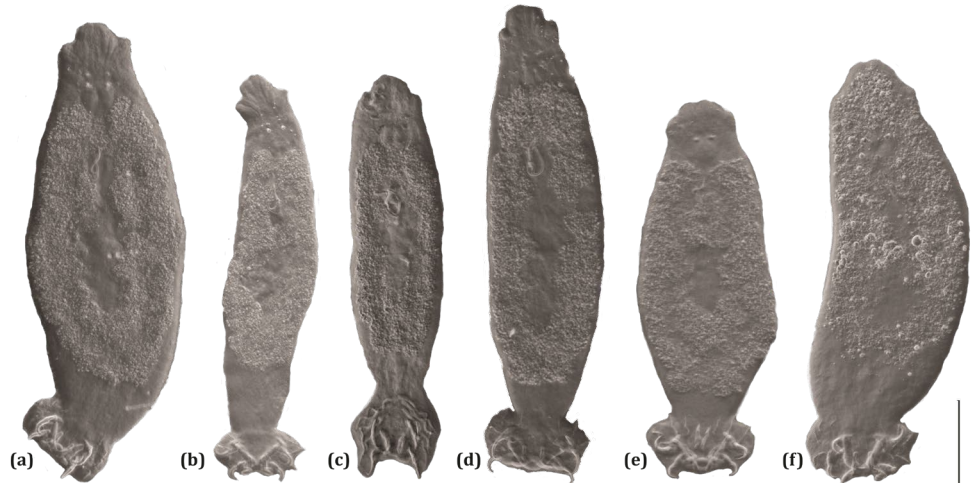

Figure 1.1
Species of monogeneans infecting the gills of sampled cichlids of southern Lake Victoria (Tanzania). **(a)** *Cichlidogyrus nyanza* n. sp., **(b)** *Cichlidogyrus furu* n. sp., **(c)** *Cichlidogyrus pseudodossoui* n. sp., **(d)** *Cichlidogyrus longipenis,* **(e)** *Cichlidogyrus vetusmolendarius* n. sp., **(f)** *Cichlidogyrus bifurcatus,* **(g)** *Gyrodactylus sturmbaueri*. Scale bar: 100 µm.

whereas adults are parasitic. *Cichlidogyrus* has been intensively studied in Tropheini of Lake Tanganyika, where it represents the most abundant and prevalent monogenean parasite (Raeymaekers et al., 2013; Grégoir et al., 2015; Vanhove et al., 2015) and probably co-diversified with their cichlid hosts (Vanhove et al., 2015). Because of their host specificity, large species number and high morphological diversity, monogeneans are good candidates for driving host diversification (Pariselle et al., 2003; Vanhove & Huyse, 2015). In this thesis, I observed six species of *Cichlidogyrus* (four of which are new species, described in **chapter 6**) and one species of *Gyrodactylus*.

Copepods – Copepods (Crustacea: Copepoda) are a common group of fish ectoparasites (Boxshall & Halsey, 2004; Luque & Tavares, 2007). Copepods display substantial morphological diversity among species. Their life cycle involves several larval stages (multiple nauplii and copepodids), which may be free-swimming or parasitic depending on the copepod species. When the last copepodid stage matures, the female copepod attaches to the final host. Adult males of most species are not parasitic but live as free swimming zooplankton.

Lamproglena monodi (Capart, 1944) is a copepod parasite apparently restricted to African cichlids, but infecting a broad range of cichlid species (Scholz et al., 2018). Recently it was accidentally introduced in Brazil together with two African cichlid species (*Oreochromis niloticus* and *Tilapia rendalli*, Azevedo et al., 2012). Females have a segmented and elongated body (3-4 mm) and, after fertilization in the water, they carry two long uniseriate egg clutches (**Fig. 1.2a**). They attach to the hosts gill filament with their maxillae, inducing local epithelium hyperplasia (Paperna, 1996). Copepodids and adult females are parasitic, whereas nauplii and adult males are free-living.

Ergasilus lamellifer (Fryer, 1961) is a copepod parasite mainly restricted to cichlids, but again infecting a broad range of species (Fryer, 1968; Scholz et al., 2018). Females have a segmented and short body (0.8-1 mm) and, after fertilization in the water, they bear two bunch-shaped egg clutches (**Fig. 1.2c**). They attach to the host's gill filament with a sharp blade-like lamella on the second pair of antennae (a distinctive trait of the species). Attachment may cause erosion and hyperplasia of the epithelium (Paperna, 1996). Only adult females are parasitic, whereas nauplii, copepodids and adult males are all free-living.

Bivalves – Several species of mollusc (Bivalvia: Unioniformes) infect the gills of fish, displaying different degrees of host specificity (Wächtler et al., 2001; Haag & Warren, 2003). Bivalves parasitizing cichlids belong to the families *Anodontidae* and *Iridinidae* (the latter exclusively infects cichlids) and to the subfamilies *Ambleminae*, *Rectidentinae* (Modesto et al., 2018). Adults are free-living. Larvae (glochidia, 0.5-2 mm) of some species have little hook(s) on their shell inner edge to attach to fish gills. Glochidia are released into the water column and need to find a suitable host within hours or days (Zimmerman & Neves, 2002). Some species search passively

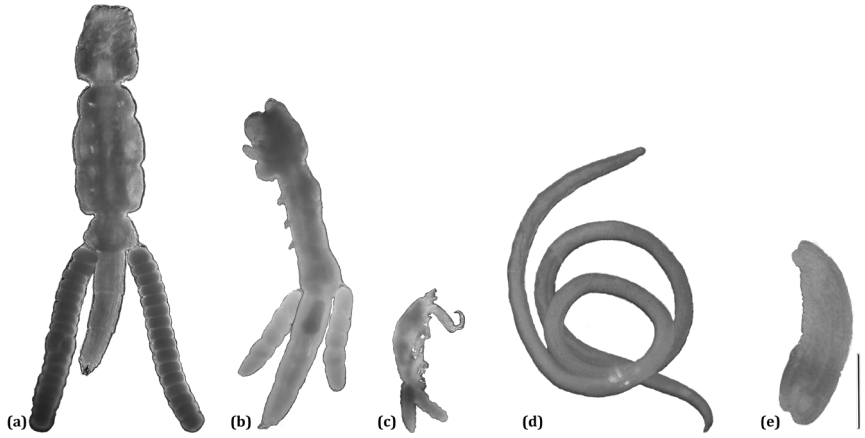

Figure 1.2
Macroparasites infecting the sampled cichlids of southern Lake Victoria, other than those belonging to *Cichlidogyrus*. Gill parasites **(a)** *Lamproglena monodi*, **(b)** *Lamproglena* spp. **(c)** *Ergasilus lamellifer* (lateral view); endoparasites **(d)** nematode, **(e)** trematode. Scale bar: 500 µm.

for a host, while others have active strategies (e.g. contractions, mucus strands, Barnhart et al., 2008). After attachment, they encyst and live on the host's body fluids (Nedeau et al., 2000) for hours or weeks depending on several factors (e.g. mussel species, host species, attachment position, water temperature; Modesto et al., 2018). They develop into juveniles that are subsequently released into the water column and will settle to become a sessile adult.

Nematodes – Most nematodes (Ecdysozoa: Nematoda) are either endoparasites of vertebrates or pathogens of plants, while some few are free-living. Freshwater fish are often infected by *Camallanoidea* and *Ascaroidea*, both having a broad host range. Most parasitic forms require one or more intermediate hosts (possibly a fish), in which larvae encyst into viscera and musculature and they moult. Infective juveniles are ingested by the final host (possibly a piscivorous bird). Adults are elongated and unsegmented roundworms (in fish: 3-80 mm; **Fig. 1.2d**). In this thesis, I do not distinguish between the genera or species because long-time dead hosts are unsuitable for reliable morphological identification of endoparasitic helminths (Scholz et al., 2018). In addition, nematodes parasitizing fish are generally generalist, hence it is less relevant for the scope of the thesis to identify them.

Trematodes – Known as flukes, trematodes (Plathyhelminthes: Neodermata) are obligate parasites, mostly endoparasites, of many vertebrates, displaying different degrees of host specificity. Their life cycle requires 1-3 intermediate hosts (the first one of which is a mollusc) and includes free-living larval stages. Adults have a flattened cylindrical body (in fish: 1-25 mm; **Fig. 1.2e**) with two muscular suckers. All species infecting African fish are hermaphrodites. In this thesis, I do not distinguish between the genera or species because long-time dead hosts are unsuitable for reliable morphological identification of endoparasitic helminths (Scholz et al., 2018).

1.4. THESIS OVERVIEW

In this thesis I investigate whether parasites initiate host speciation or contribute to host species divergence after speciation or neither, by analysing the macroparasite infection of cichlids from Lake Victoria. In **chapter 2 and 5**, I studied a large sympatric cichlid fish community that included 17 species of the Lake Victoria radiation and two species only distantly related to the radiation that represent two distinct haplochromine lineages that never speciated in this area despite a long evolutionary history in the lake region (*Astatoreochromis alluaudi*, *Pseudocrenilabrus multicolor victoriae*). In **chapter 3 and 4**, I focused on species pairs of *Pundamilia* that vary in their age since speciation and the extent of genetic differentiation. I included sympatric forms with blue or red male nuptial coloration from four locations: an old species pair at Makobe Island that is genetically strongly differentiated and shows no evidence of recent genetic exchange; a young species pair at Python and Kissenda Islands, that are genetically differentiated and mate assortatively but have some low level of gene flow; a single panmictic population with blue, red and intermediate male colour morphs at Luanso Island (**Fig. 1.3**).

Fish were found to be infected by five ectoparasite genera on the gills (*Cichlidogyrus* spp., *Gyrodactylus sturmbaueri*, *Lamproglena monodi*, *Ergasilus lamellifer*, glochidia larvae of bivalves) and two types of endoparasites in the abdominal cavity (nematodes, trematodes) (**Fig. 1.1** and **1.2**). The flatworm genus of *Cichlidogyrus* is particularly promising to study the link between parasites and host diversification, because it is a species-rich genus with high morphological diversity, display high host specificity and it co-evolved with cichlids in at least one other African lake (Pariselle et al., 2003; Vanhove et al., 2016). Therefore, I also identified *Cichlidogyrus* to species level based on the morphology of male copulatory organ and attachment organ. I found *C. longipenis* Paperna & Thurston 1969 and *C. bifurcatus* Paperna 1960 (redescribed in **chapter 6**) and four new species: *Cichlidogyrus furu, C. nyanza, C. vetusmolendarius, C. pseudodossoui* (described in **chapter 6**). Species of *Cichlidogyrus* were provisionally named with roman numbers in papers published before the formal taxonomic description (**Gobbin et al., 2020b**; **Gobbin et al., 2021**). For the sake of consistency and clarity, I use the new species names throughout the thesis (**Table 1.1**).

Results differed according to the infection level considered: *i)* between parasites of higher taxonomic levels (hereafter referred to as parasite higher taxon level) and *ii)* between species of *Cichlidogyrus* (hereafter referred to as *Cichlidogyrus* species level).

In **chapters 2 and 3**, I compared parasite infection among host species in two sampling years. I found that two prerequisites of parasite-mediated speciation are met (Karvonen & Seehausen, 2012): reproductively isolated host species differ in parasite infection and the direction of

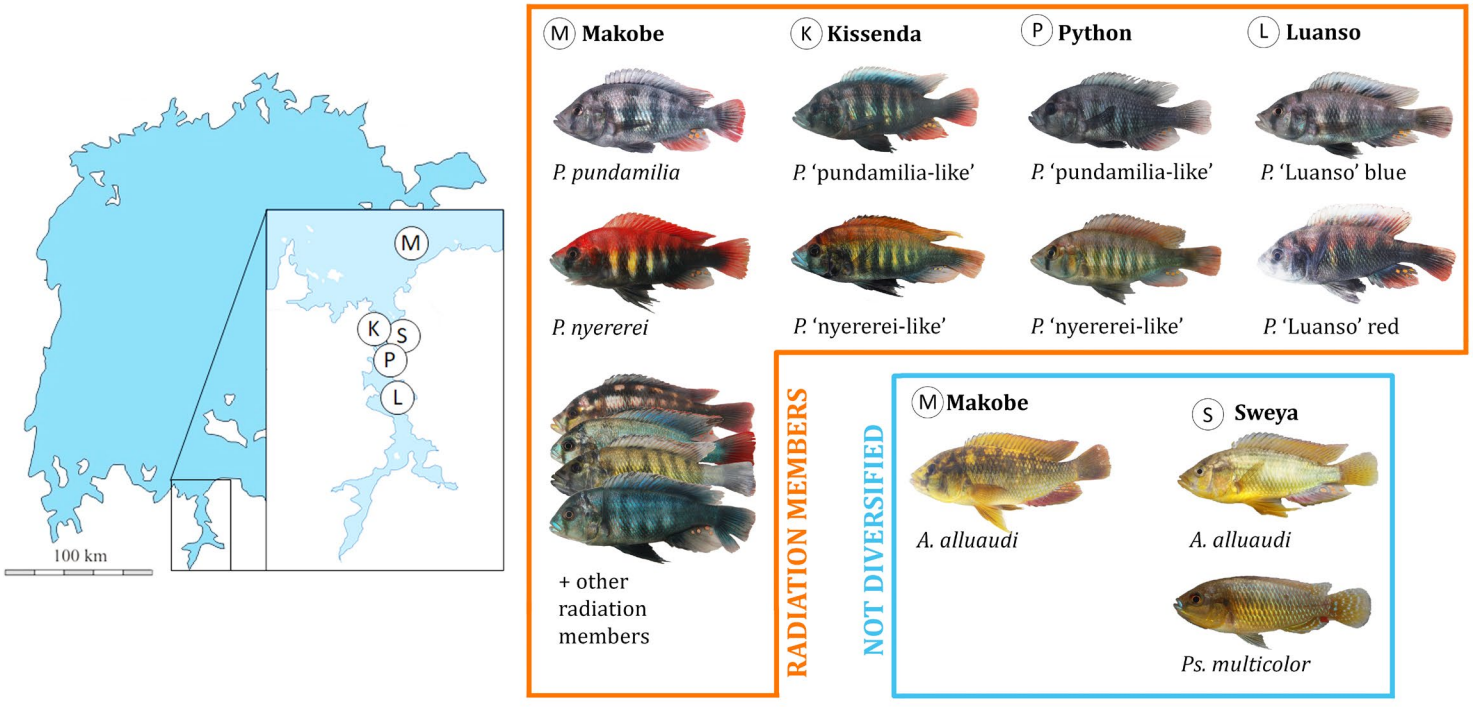

Figure 1.3
Sampling sites in southern Lake Victoria, Tanzania: rocky islands Makobe (M), Kissenda (K), Python (P), Luanso (L) and the Sweya swampy inlet stream (S). For each location, sampled cichlid species are depicted (orange frame: radiation lineage, blue frame: two lineages that did not diversified).

parasite-mediated selection is consistent over time. I also found that variation between species is partially explained by habitat and trophic ecology, whereas the remaining variation might be explained by intrinsic differences between species (i.e. immunity).

In **chapter 3**, I also investigated whether divergence in infection between male colour morphs is already present *before* measurable genetic differentiation at neutral markers, which would be consistent with a role for parasites in the initiation of a speciation process (rather than following it). At parasite higher taxon level, the extent of parasite community dissimilarity increased with increasing genetic distance among sympatric host species; whereas the dissimilarity in the *Cichlidogyrus* species assemblage did not correlate with host genetic distance. This suggests that differences in infection with different parasite genera (but not with different species of *Cichlidogyrus*) may contribute to divergent selection between already differentiated host species, but there is no evidence that differences in infection precede species differentiation as would be expected if they were initiating speciation it.

In **chapter 4**, I assessed the contribution of extrinsic (exposure) and intrinsic factors (genetically based resistance) to host species differences in infection. I compared the infection patterns between two closely related sympatric blue and red species of *Pundamilia*, using wild-caught and first-generation lab-reared fish, as well as lab-reared interspecific hybrids. Species differences in infection as observed in the wild were not maintained under laboratory conditions with standardized exposure, suggesting that differences in immune traits had not yet evolved in a young sympatric species pair. This does not support the idea that parasites mediate divergence during speciation in *Pundamilia*.

In **chapter 5**, I investigated additional axes of infection variation among cichlid species. I observed differences between host species in the non-random microhabitat distribution of parasites on the gills, indicating species-specificity in niche selection, consistently with parasite-mediated diversification. Parasite-parasite relationships (positive at parasite higher taxon level and negative at *Cichlidogyrus* species level) and copepod reproductive activity did not differ between host species, indicating no specificity of the host-parasite relationships.

Table 1.1
Provisional and formal species names of *Cichlidogyrus*. Species of *Cichlidogyrus* are (re)described in **chapter 6**. Before the formal taxonomical description, these species were provisionally named with roman numbers

Provisional name	New name
Cichlidogyrus sp. I	*Cichlidogyrus nyanza* n. sp.
Cichlidogyrus sp. II	*Cichlidogyrus furu* n. sp.
Cichlidogyrus sp. III	*Cichlidogyrus pseudodossoui* n. sp.
Cichlidogyrus sp. IV	*Cichlidogyrus longipenis*
Cichlidogyrus sp. V	*Cichlidogyrus vetusmolendarius* n. sp.
Cichlidogyrus sp. VI	*Cichlidogyrus bifurcatus*

BOX I – GLOSSARY

Bray-Curtis distance: a quantitative measure of dissimilarity, here used to quantify the differences in parasite abundance between host species.

Divergent selection: selection acting in contrasting directions within each of several populations (e.g. large size favoured in one population, small size in another). It is considered ecological when the agents of selection are environment-dependent (e.g. large size favoured in meadow, small size in wood).

Ecological speciation: mechanism of speciation in which reproductive isolation between populations is caused by ecologically based divergent selection (e.g. divergent parasite infections).

Exposure: the extent to which the host encounters the parasite, determined by host ecology (i.e. diet, habitat), parasite ecology and parasite absolute numbers.

Gill filament: one of the numerous filamentous processes forming the comb-like structure of a gill arch. Each gill arch is composed by two parallel sets of filaments. Each gill filament is folded into numerous secondary lamellae, to increase the gill surface for gas exchanges. Also referred to as **primary lamellae**.

Gill microhabitat: artificial categories in which the gills are subdivided. In this thesis, to explore potential spatial niche segregation, I considered the following gill subdivisions: 36 microhabitat sites, four gill arches, three longitudinal segments (dorsal, median, ventral), three vertical areas (proximal, central, distal) (**Fig. 5.1a**).

Haptor: the attachment organ of the monogeneans. Here, refers to the posterior haptor (opisthaptor) consists of sclerotized hooklets and uncinuli that allow firm attachment on the gill filament. The morphology of opisthaptor and of male copulatory organ are used by taxonomists to discriminate species.

Host specificity: the extent to which a parasite taxon is restricted in the number of host species used at a given stage in the life cycle (Poulin, 2007). Host specificity decreases as the number of host species increases.

Infection levels: a quantitative measure of infection, that refers to parasite prevalence, abundance or intensity.

Intensity of infection: number of individuals of a given parasite taxon in/on a given host individual.

Jaccard similarity index: a qualitative measure of dissimilarity, here used to quantify the differences in parasite diversity (presence/absence of parasite species) between host species.

Mean intensity of infection: is the average number of individuals of a given parasite taxon over all infected hosts in the sample in a given host species or population. In contrast to parasite abundance, intensity includes only infected host individuals.

Parasite abundance: the average number of individuals of a given parasite taxon per host individual in a given host species or population. It includes both infected and uninfected host individuals.

Parasite community composition: a measure of community structure that takes into account presence/absence of parasite species and the numbers of individuals belonging to each parasite species infecting a given host species or population. Also referred to as **infection profile**.

Parasite-mediated speciation: the process in which divergent adaptation to parasites leads to speciation of the host.

Parasite prevalence: the proportion (usually expressed as percentage) of hosts of a given species or population that are infected by a given parasite taxon.

Reproductive isolation: decreased probability of successful breeding between members of two species or populations. It can arise from prezygotic and/or postzygotic mechanisms.

Resistance: ability of a host to limit the parasite intensity. This can be achieved through immune defences (which we mostly refer to in this thesis) or by parasite avoidance. It has a negative effect on parasite survival and reduces parasite intensity and prevalence in a host population (which may result in a negative feedback loop: a decrease in parasite prevalence will reduce the fitness advantage of having the resistance; Roy & Kirchner, 2000).

Speciation: process in which inbreeding populations evolve reproductive isolation, thereby diverging into two or more species.

Susceptibility: a predisposition to become infected, given exposure. It arises from the interaction of host genetic and environmental factors (e.g. nutritional status, concomitant diseases).

Tolerance: ability of a host to limit the fitness costs induced by a given parasite intensity. It does not have direct negative effects on the parasite survival and can have neutral or positive effect on parasite prevalence.


2

2

Temporally consistent species differences in parasite infection but no evidence for rapid parasite-mediated speciation in Lake Victoria cichlid fish

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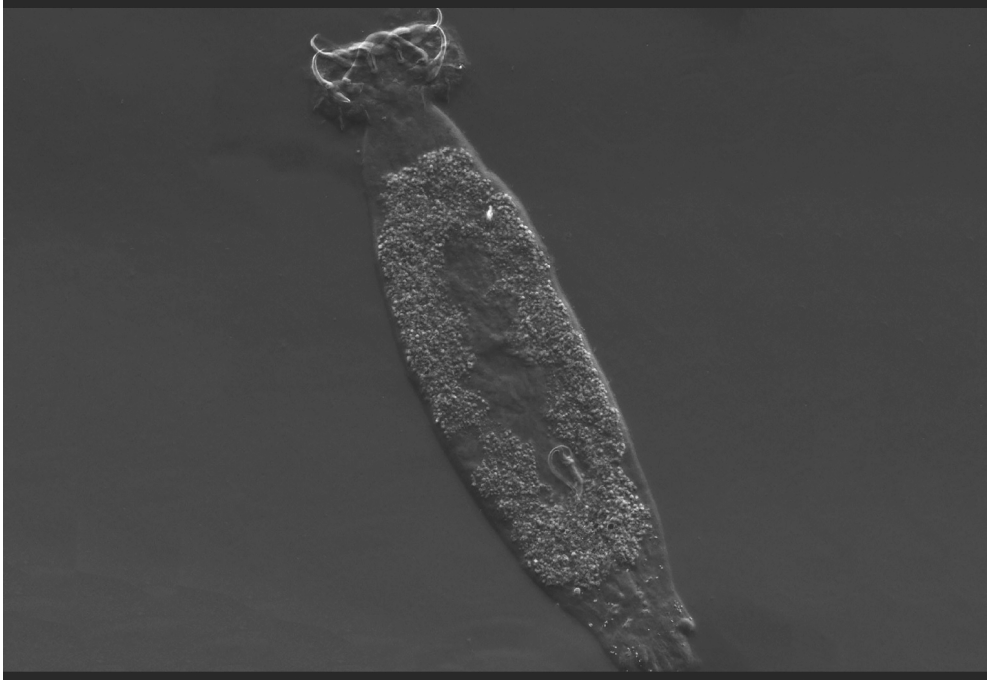

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ABSTRACT

Parasites may have strong eco-evolutionary interactions with their hosts. Consequently, they may contribute to host diversification. The radiation of cichlid fish in Lake Victoria provides a good model to study the role of parasites in the early stages of speciation.

We investigated patterns of macroparasite infection in a community of 17 sympatric cichlids from a recent radiation and 2 older species from 2 non-radiating lineages, to explore the opportunity for parasite-mediated speciation. Host species had different parasite infection profiles, which were only partially explained by ecological factors (diet, water depth). This may indicate that differences in infection are not simply the result of differences in exposure, but that hosts evolved species-specific resistance, consistent with parasite-mediated divergent selection. Infection was similar between sampling years, indicating that the direction of parasite-mediated selection is stable through time.

We morphologically identified 6 *Cichlidogyrus* species, a gill parasite that is considered a good candidate for driving parasite-mediated speciation, because it is host species-specific and has radiated elsewhere in Africa. Species composition of *Cichlidogyrus* infection was similar among the most closely related host species (members of the Lake Victoria radiation), but two more distantly related species (belonging to non-radiating sister lineages) showed distinct infection profiles. This is inconsistent with a role for *Cichlidogyrus* in the early stages of divergence.

To conclude, we find significant interspecific variation in parasite infection profiles, which is temporally consistent. We found no evidence that *Cichlidogyrus*-mediated selection contributes to the early stages of speciation. Instead, our findings indicate that species differences in infection accumulate after speciation.

Keywords:

parasite-mediated selection, diversification, adaptive radiation, host-parasite interaction, Cichlidae

2.1. INTRODUCTION

Ecological speciation, the evolutionary process by which ecologically-based divergent selection leads to species divergence, can be driven by adaptation to both abiotic and biotic factors. Antagonistic interactions among species (i.e. prey-predator, resource competition) are commonly considered examples of biotic factors that may drive ecological speciation (Schluter, 1996, 2000b; Rundle & Nosil, 2005; Maan & Seehausen, 2011).

Parasites form another ubiquitous selective pressure (Poulin & Morand, 2000; Schmid-Hempel, 2013) and engage with their hosts in coevolutionary dynamics of adaptation and counter-adaptation (Decaestecker et al., 2007). Heterogenous parasite-mediated selection, as different infection levels of a parasite species and/or different parasite community compositions may initiate, promote or reinforce host diversification and ecological speciation. Studies investigating the role of parasites in host diversification have begun to accumulate (Greischar & Koskella, 2007; Eizaguirre et al., 2011; Eizaguirre et al., 2012a; Stutz et al., 2014; Feulner et al., 2015; Karvonen et al., 2015). However, parasite-mediated selection has received relatively little attention in the context of adaptive radiation (Vanhove & Huyse, 2015; El Nagar & MacColl, 2016).

Adaptive radiations are characterized by the rapid evolution of ecologically distinct taxa in response to new ecological opportunities or challenges (Schluter, 2000b; Rundle & Nosil, 2005). Parasites may contribute to this process if three prerequisites are met (Rundle & Nosil, 2005; Karvonen & Seehausen, 2012). First, parasite-mediated selection should differ within or between host populations in terms of parasite abundance and/or community composition. Consistent with this, previous studies have reported infection differences among closely related host species across a wide range of animal taxa (mammals: Boundenga et al., 2018; reptiles: Carbayo et al., 2018; fish: Thomas et al., 1995; MacColl, 2009a; bivalves: Coustau et al., 1991; crustaceans: Galipaud et al., 2017). Second, parasitic infection should impose a cost on host fitness, thereby exerting selection for resistance or tolerance on the host. This prerequisite is also supported by empirical evidence from a wide range of taxa (mammals: Careau et al., 2010; fish Milinski & Bakker, 1990; crustaceans: Stirnadel & Ebert, 1997; Tellenbach et al., 2007; angiosperms: Segar et al., 2018; birds: Hamilton & Zuk, 1982). Third, the direction of parasite-mediated selection between host populations should be stable over time. Stochastic or frequency-dependent temporal fluctuations in parasite abundances could cause variation in the strength of parasite-mediated selection, but the direction of divergent selection is stable if the differences between host populations in parasite exposure or impact are maintained. Temporally consistent infection differences have been observed in cichlids of Lake Tanganyika (Raeymaekers et al., 2013) and in icefish from the Antarctic Sea (Mattiucci et al., 2015). In response to parasite-mediated divergent

selection, host (sub)populations may adapt either by evolving a specialised immune response or by evolving increased tolerance (depending on their respective costs and benefits). Such adaptive responses can lead to an increasingly different parasite infection pattern between host (sub)populations. Here we investigate two prerequisites of parasite-mediated speciation in the same study system, by analysing infection differences – in terms of parasite communities and individual parasite taxa – between several sympatric host species within an adaptive radiation of cichlid fish, at two different time points.

Parasite transmission is associated with specific habitats and foraging strategies; therefore, host populations with different ecological specializations may encounter different parasites, even in geographic sympatry (Hablützel et al., 2017; Hayward et al., 2017). Host populations that are exposed to different parasites are expected to respond to parasite-mediated divergent selection, potentially strengthening host species differentiation. According to the hybrid/immigrant disadvantage hypothesis (Fritz et al., 1994), hybrids between two diverging host populations may not cope well with the infection of either parental species because of their recombinant resistance genotype. For example, hybrids may have a super-optimal MHC diversity, causing a reduced T-cell repertoire (through elimination of T-cells that are binding self-peptides; Janeway et al., 2005) and making them more susceptible to parasites (Eizaguirre et al., 2012a). As a result, parasite-mediated selection against recombinants can reduce geneflow between parental species. Alternatively, the recombinant resistance genotype of hybrids outperforms parental resistance genotypes (Baird et al., 2012). In that case, parasite-mediated selection could promote geneflow and reduce the opportunity for speciation. Since specific MHC alleles may confer resistance to specific parasites (Paterson et al., 1998; Bonneaud et al., 2006; Eizaguirre et al., 2009b), both scenarios may occur at the same time: for some infections, recombinants are favoured, but not for others.

Cichlid fish of the Great African Lakes (Lakes Malawi, Tanganyika and Victoria) are a well-studied example of adaptive radiation (Kornfield & Smith, 2000; Kocher, 2004; Seehausen, 2006). At the same time, cichlids also provide many examples of no diversification, as most lineages never radiated into multiple species despite extensive ecological opportunity (Seehausen, 2015). Within radiations, the Lake Victoria rock cichlids are a classical example of species divergence in macro-habitat, micro-habitat and trophic specialization (Bouton et al., 1997; Seehausen & Bouton, 1997; Seehausen & Bouton, 1998). This suggests that they may be exposed to different parasite taxa (Maan et al., 2008; Karvonen et al., 2018) and thus good candidates for responding to parasite-mediated divergent selection.

Here, we investigate the potential role of parasites in host diversification by analysing macroparasite infection in Lake Victoria cichlid fish. In addition to higher taxon-level identification, we assess morphospecies diversity of *Cichlidogyrus*, a genus of flatworm gill parasites (Monogenea, Ancyrocephalidae) that primarily infects members of the Cichlidae family

(but also killifishes belonging to *Aphyosemion*, Messu Mandeng et al., 2015, and the nandid *Polycentropsis abbreviata*, Pariselle & Euzet, 2009). *Cichlidogyrus* is the most species-rich parasite taxon infecting old world cichlids (Scholz et al., 2018), and has undergone at least one radiation (in Lake Tanganyika, Vanhove et al., 2015). Host specificity of representatives of *Cichlidogyrus* has been observed in Lake Tanganyika, but is poorly investigated in other lakes (Pariselle et al., 2015). Recent studies experimentally confirmed that monogeneans cause an immune response in their host (Zhi et al., 2018; Chen et al., 2019), providing evidence for the second prerequisite for parasite-mediated speciation. Together, the often relatively high host specificity, large species number and high morphological diversity within the genus, make *Cichlidogyrus* a good model to study the evolution of host-parasite interactions (Pariselle et al., 2003; Vanhove et al., 2016).

In a previous study, ectoparasite infections in a cichlid fish species assemblage of a rocky island in Lake Victoria were found to differ between host species, and to be correlated with host species differences in water depth occupation, diet and abundance (Karvonen et al., 2018). Here, we study the same assemblage, allowing us to test the temporal consistency in these patterns. We also expand on the earlier findings by including endoparasites and by identifying monogenean parasites to species level. We expect divergent infections between host species of the radiation, in both parasite community composition and parasite abundance, in line with the first prerequisite for parasite-mediated speciation. Moreover, parasite-mediated selection should generate species differences in infection that are not explained by ecological factors alone. If variation in parasite infection across host species is fully explained by variation in host capture depth and diet, it could be driven entirely by environmental variation in exposure, and would not constitute evidence for divergent evolution of host-specific defence mechanisms. Following the third prerequisite for parasite-mediated speciation, we also expect that the direction of infection differences between host species is constant through time, thus maintaining the direction of divergent selection even in the presence of temporal fluctuations in parasite abundances.

We include two cichlid species (*Astatoreochromis alluaudi* and *Pseudocrenilabrus multicolor*) that have not been investigated previously for their *Cichlidogyrus* infection. They are not part of the radiation of cichlids in Lake Victoria and only distantly related to the radiation (Schedel et al., 2019), yet they co-occur with the radiation cichlids. If parasite-mediated selection contributed to the Lake Victoria cichlid radiation, we predict that radiation members have adapted to parasites by evolving specific immune responses, whereas these two older lineages that did not diversify in response to parasites (nor to other factors), evolved an unspecialised defence (i.e. generalist tolerance or resistance). This would result in different infection patterns, possibly characterised by higher within-host parasite diversity (more species of *Cichlidogyrus*) and parasite abundance (more individuals of *Cichlidogyrus*) in the non-diversifying lineages. Variation in infection patterns of *Cichlidogyrus* within and between cichlid lineages could emerge from at

least two evolutionary scenarios. First, worms colonized the radiation cichlids from the ancient non-radiating cichlids, with different worm species colonizing the differentiating hosts in different numbers. This would impose different selection pressures on different host species and could initiate host-specific evolutionary responses. This scenario would lead to a pattern in which *Cichlidogyrus* species are shared among the radiation cichlids and the older, non-radiating lineages. Alternatively, ancestral worms may have diverged after colonizing the radiation cichlids, co-speciating with their hosts. This latter pattern, with *Cichlidogyrus* species not shared between radiation members and the older non-radiating lineages, would support a contribution of *Cichlidogyrus*-mediated selection to the Lake Victoria cichlid radiation.

2.2. METHODS

2.2.1. Fish collection

Cichlid fish were collected in May-August 2010 at Makobe Island and in June-October 2014 at three locations in southern Lake Victoria, Tanzania (Makobe Island, Sweya swamp and Kissenda Island, **Fig. 2.1**). At Makobe, we collected 18 sympatric cichlid species representing different ecological specializations (diet and water depth, Witte & van Oijen, 1990; Seehausen, 1996b; Bouton et al., 1997; Seehausen & Bouton, 1998; **Table 2.1**), and also different levels of genetic differentiation (Wagner et al., 2012b; Karvonen et al., 2018). Of those, 17 species belong to the Lake Victoria radiation and one species (*Astatoreochromis alluaudi*) represents an old lineage that has not radiated. Since Makobe is inhabited by only one of the two non-radiating haplochromine species that occur in Lake Victoria, it was necessary to sample a second location, Sweya, to obtain the other one (*Pseudocrenilabrus multicolor*). The divergence between the two non-radiating species, and between them and the ancestors of the radiations in Lake Victoria, Lake Malawi and other lakes, dates back to ~15 million years ago (Schedel et al., 2019). Including Sweya introduced geographical variation as an additional variable. To assess the effects of geographical distance on parasite infection patterns, we therefore also collected additional specimens of *A. alluaudi* from this second location (Sweya). For the same reason, we also added a third location, the rocky island Kissenda, where we sampled two species of the radiation (*P.* sp. 'pundamilia-like' and *P.* sp. 'nyererei-like'), that are closely related and ecologically similar to two Makobe species (*P. pundamilia* and *P. nyererei* respectively). Finally, to increase the number of molluscivore species, we also sampled *Ptyochromis xenognathus* (belonging to the radiation) at Kissenda.

Collection was done by angling and with gillnets of variable mesh sizes, set at different water depths (0-19 m). Males and females may differ in infection pattern (Maan et al., 2006b). However, females are difficult to identify reliably in the field, due to their generally cryptic

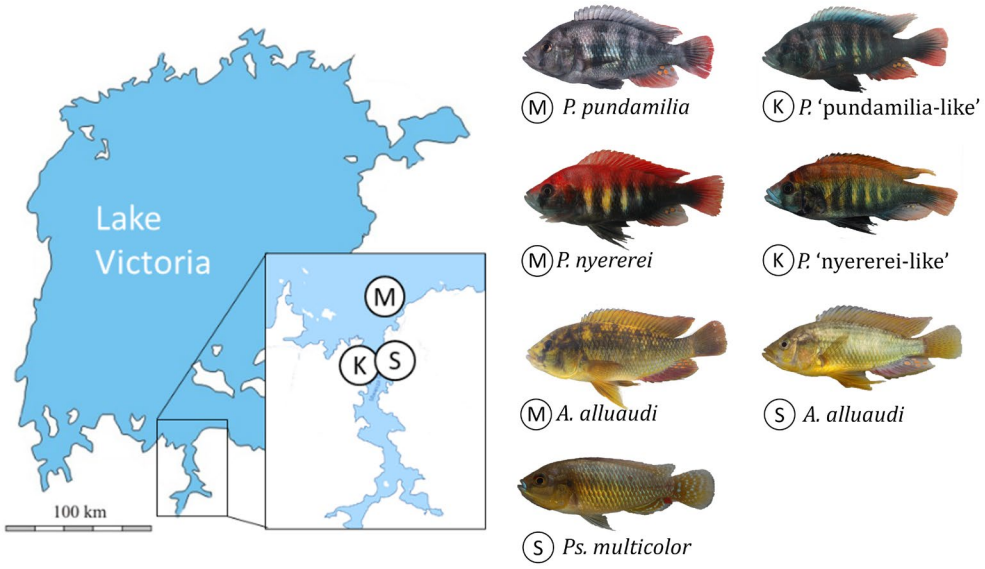

Figure 2.1
Geographical location of the three sampling sites in southern Lake Victoria, Tanzania: rocky islands Makobe (M) and Kissenda (K) and the Sweya swampy inlet stream (S). Depicted are the two non-radiating lineages, represented by *Astatoreochromis alluaudi* (collected from both Makobe and Sweya) and *Pseudocrenilabrus multicolor* (collected from Sweya); as well as representatives of the radiation: two closely related species pairs collected from Makobe (*Pundamilia pundamilia*, *P. nyererei*) and at Kissenda (*P.* sp. 'pundamilia-like', *P.* sp. 'nyererei-like').

coloration. We therefore included only males. Fish were euthanised with an overdose of 2-phenoxyethanol immediately after capture. Their body cavity was slit open ventrally to allow preservation of organs and internal parasites. Some fish were preserved in 4% formalin and subsequently transferred on 70% ethanol, other fish were directly preserved in 100% ethanol for future genetic analysis. Each individual fish was subsequently measured (SL standard length, BD body depth, to the nearest 0.1 mm) and weighed (to the nearest 0.1 g).

2.2.2. Parasite screening

We examined gill arches (right side of the fish only), abdominal cavity, gonads, liver and gastrointestinal tract under a dissecting stereoscope. All macroparasites were identified following Paperna (1996 and monogenean literature (Vanhove et al., 2011; Muterezi Bukinga et al., 2012; Zahradníčková et al., 2016) and counted. Five ectoparasite taxa and two endoparasite taxa were found. Encysted skin trematodes of the 'Neascus' type (Paperna, 1996) were not included because consistency of detection was low due to their cryptic appearance. All monogenean worms infecting gills were individually preserved in 100% ethanol. With the

exception of one individual of *Gyrodactylus* sp., these all belonged to *Cichlidogyrus*. For morphological identification we selected a subset of *Cichlidogyrus* specimens (n=640) from 17 host species (the two species from the two non-radiating lineages, 15 species from the radiation). We aimed to identify 15 *Cichlidogyrus* specimens per host population, by sampling all worms infesting each fish individual from a randomly selected pool of each host population. If the total number of worms available per host population was less than 15, then all worms of that host population were identified (see **Table 2.1** for sample sizes).

2.2.3. *Cichlidogyrus* species identification

For morphological analysis, specimens of *Cichlidogyrus* were mounted on slides in Hoyer's medium, after prior treatment with 20% sodium dodecyl sulphate to soften tissues. Specimens of *Cichlidogyrus* were examined with a microscope (Olympus BX41TF) under 1000x magnification using differential interference phase contrast. Species of *Cichlidogyrus* were discriminated based on shape and size of sclerotized parts of the attachment organ (haptor) and, in particular, on those of the male copulatory organ (MCO) (e.g. Grégoir et al., 2015).

Table 2.1
Characteristics of host species sampled in 2014 at Makobe, Sweya and Kissenda islands: diet, number of fish individuals, water depth, SL standard length, weight, CF condition factor. Species labelled with a circle (●) were also sampled in 2010 (only sample sizes reported, other data available in Karvonen et al., 2018), and those with a square (■) were used to assess *Cichlidogyrus* diversity (number of identified worm specimens reported, N id *C.*).

	Host species	Diet	N fish 2014	N id *C.*	Depth (m)		SL (mm)		Weight (g)		CF		N fish 2010
					mean	(min-max)	mean	(min-max)	mean	(min-max)	mean	(min-max)	
Makobe													
■●	*Astatoreochromis alluaudi*	mollusc	17	38	9.6	(0.75-18.5)	111.28	(70.9-130.8)	46.59	(10.8-71.5)	3.09	(2.72-3.46)	10
	Haplochromis serranus	fish	2		15.0	(11.0-19.0)	133.29	(125.3-141.3)	68.54	(68.5-68.5)	2.32	(2.20-2.43)	0
■●	*Labrochromis* sp. 'stone'	mollusc	1	3	19.0	(19.0-19.0)	130.75	(130.8-130.8)	65.45	(65.5-65.5)	2.84	(2.84-2.84)	14
●	*Lipochromis melanopterus*	fry	2		8.8	(5.5-12.0)	91.96	(80.8-103.1)	24.76	(16.5-33.0)	2.94	(2.90-2.99)	8
	Lipochromis sp. 'yellow chin pseudonigricans'	insect	10		11.0	(9.0-19.0)	92.05	(79.7-113.0)	34.57	(21.3-47.9)	2.52	(2.23-3.26)	0
■●	*Mbipia lutea*	algae	7	14	1.0	(1.0-1.0)	139.68	(136.0-142.0)	76.87	(67.1-83.4)	2.81	(2.56-3.08)	13
■●	*Mbipia mbipi*	algae	16	22	1.9	(1.0-2.5)	97.33	(84.7-113.2)	30.31	(20.3-40.5)	2.87	(2.54-3.72)	16
■●	*Neochromis gigas*	algae	8	15	1.2	(1-2.75)	114.99	(86.2-127.3)	43.11	(17.9-52.4)	2.75	(2.52-2.94)	13
■●	*Neochromis omnicaeruleus*	algae	26	25	4.8	(2.5-9.5)	91.86	(74.0-110.5)	23.78	(11.3-41.6)	2.82	(2.28-3.54)	9
■●	*Neochromis rufocaudalis*	algae	16	13	2.6	(0.75-3.5)	89.21	(61.4-100.0)	20.28	(6.4-26.3)	2.70	(2.41-3.08)	9
■●	*Neochromis* sp 'unicuspid scraper'	algae	32	23	13.2	(1.25-19.0)	96.73	(76.6-114.4)	26.16	(10.9-49.4)	2.69	(2.19-3.21)	8
■●	*Pundamilia nyererei*	plankton	71	34	10.6	(2.5-18.5)	81.28	(63.0-106.7)	17.69	(7.0-41.9)	2.74	(2.06-3.41)	10
■●	*Pundamilia* sp. 'pink anal'	plankton	18	15	9.9	(5.5-19.0)	91.79	(77.9-120.8)	24.78	(12.2-59.1)	2.80	(2.37-3.43)	10
■●	*Pundamilia pundamilia*	insect	56	21	1.7	(0.5-16.0)	95.32	(52.1-128.8)	33.54	(3.7-71.3)	3.15	(2.50-3.76)	9
■●	*Paralabidochromis chilotes*	insect	9	5	12.3	(1.5-19.0)	106.35	(81.1-120.8)	47.13	(34.1-53.7)	2.46	(2.09-2.95)	11
■●	*Paralabidochromis cyaneus*	insect	14	16	2.7	(1-6.5.0)	100.16	(81.4-107.9)	24.43	(12.3-33.7)	2.32	(2.08-2.63)	9
●	*Paralabidochromis sauvagei*	insect	11		7.5	(3.5-14.0)	103.18	(93.7-115.4)	30.74	(11.3-44.8)	2.76	(1.06-3.42)	11
●	*Paralabidochromis* sp. 'short snout scraper'	algae	11		4.6	(3.0-6.0)	105.31	(93.5-115.5)	37.32	(22.8-44.8)	3.04	(2.70-3.29)	9

Table 2.1. (continued)

Host species	Diet	N fish 2014	N id C.	Depth (m)		SL (mm)		Weight (g)		CF		N fish 2010
				mean	(min-max)	mean	(min-max)	mean	(min-max)	mean	(min-max)	
Sweya												
■ *Astatoreochromis alluaudi*	mollusc	6	19	0.5	(0.5-0.5)	63.63	(48.2-80.3)	8.85	(2.9-15.6)	2.89	(2.50-3.26)	0
■ *Pseudocrenilabrus multicolor*	insect	20	12	0.5	(0.5-0.5)	39.60	(32.8-46.8)	1.94	(1.1-2.7)	3.01	(2.19-3.86)	0
Kissenda												
■ *Pundamilia* sp. 'nyererei-like'	insect	32	6	4.2	(0.75-7.5)	73.42	(60.1-88.9)	11.56	(4.8-26.7)	2.68	(1.92-3.68)	0
■ *Pundamilia* sp. 'pundamilia-like'	insect	31	13	3.0	(0.75-7.5)	76.21	(49.3-108.1)	13.96	(2.8-38.5)	2.58	(1.58-3.46)	0
■ *Ptyochromis xenognathus*	mollusc	0	18	3.0	(1.5-7.0)	107.76	(97.4-115.4)	37.39	(29.8-44.9)	2.93	(2.63-3.16)	10

2.2.4. Data analysis

Divergent parasite infection

To compare parasite communities between host species inhabiting Makobe Island, we performed one-way analysis of similarities, based on the zero-adjusted Bray-Curtis distances of parasite abundance data (i.e. the number of parasites in infected and uninfected host individuals) and on the Jaccard index of presence/absence of parasite species (ANOSIM, 9999 permutations, PAST 3.18, Hammer *et al.* 2001). Pairwise comparisons were made using the false discovery rate correction for P values (Benjamini & Hochberg, 1995). Such analyses were performed on fish individuals for which we established both endo- and ectoparasite infection (2014 only; fish were not screened for endoparasites in 2010) and on fish individuals for which we established ectoparasite infection in both years (2014 and 2010). To evaluate the extent to which these differences could be explained by differences in diet or depth habitat, we performed PERMANOVA (PAST). Since PERMANOVA considers categorical variables, individual capture depths were categorized into depth ranges of different resolution (1 m, 2 m, 3 m, 5 m, 10 m). To investigate the contribution of each parasite taxon to parasite community differences, similarity percentages analysis (SIMPER, PAST) was performed (reported in Supplementary Material).

Ectoparasite (pooling all species of *Cichlidogyrus*) and endoparasite taxa infecting the Makobe cichlid community in 2014 were analysed separately for prevalence (percentage of infected individuals of total host population) and infection intensity (number of parasites per infected individual), using generalized linear models in R (3.4.1. R Core Team 2018) with binomial distribution for prevalence and Poisson distribution for intensity. Fixed effects included host species, individual capture water depth and diet. Fish standard length was not included because its correlation with infection was inconsistent across species (**Fig. S2.1**). However, to account for the effect of fish length in species variation in parasite infection, we performed an additional analysis that included fish standard length as a fixed effect. We determined the significance of fixed effects by likelihood ratio tests (LRT) to select the Minimum Adequate Model (MAM). The MAM was confirmed by bootstrapping (*bootStepAIC* package). We then used model comparison to test the MAM against models including the removed terms (LRT bootstrap and Akaike Information Criterion) to obtain parameter estimates for all terms.

Temporal consistency of infection

To investigate temporal consistency in infection, we compared ectoparasite infection profiles (endoparasites were not assessed in 2010) for 16 of the 18 host species from Makobe between samples collected in 2014 and samples collected in 2010 at the same location (from Karvonen *et al.* 2018), using ANOSIM as described above. For each ectoparasite taxon, we performed generalized linear models on parasite prevalence and intensity (both years) to assess temporal

consistency. Fixed effects included host species, diet, individual capture water depth, sampling year and the interaction between sampling year and host species. Fish standard length was not included in the model, because species differences in fish length were consistent between the two years (**Fig. S2.2**) and because its correlation with infection was inconsistent across species (**Fig. S2.1**).

We also assessed temporal consistency of parasite-mediated divergent selection within pairs of closely related species (following Seehausen, 1996b; Magalhaes et al., 2012; Keller et al., 2013; Wagner et al., 2013; Brawand et al., 2014). We plotted the mean infection intensity and prevalence in 2014 against that in 2010 (**Fig. S2.3**, **S2.4**), then we established the slope of the line connecting the two species (for species pairs) and the slope of the correlation for all species (for the community-level analysis). A positive correlation slope would indicate temporal consistency in infection differences.

Divergent parasite infection at Cichlidogyrus *species level*

Differences between host species of the radiation in the community composition of *Cichlidogyrus* species were analysed using ANOSIM as described above. Pairwise comparisons were made using the false discovery rate correction for P values (Benjamini & Hochberg, 1995). The same analysis was performed to compare communities of *Cichlidogyrus* between the three haplochromine lineages (radiation members, *A. alluaudi*, *Ps. multicolor*). To investigate the contribution of each species of *Cichlidogyrus* to parasite community differences, similarity percentages analysis (SIMPER, PAST) was performed (reported in Supplementary Material).

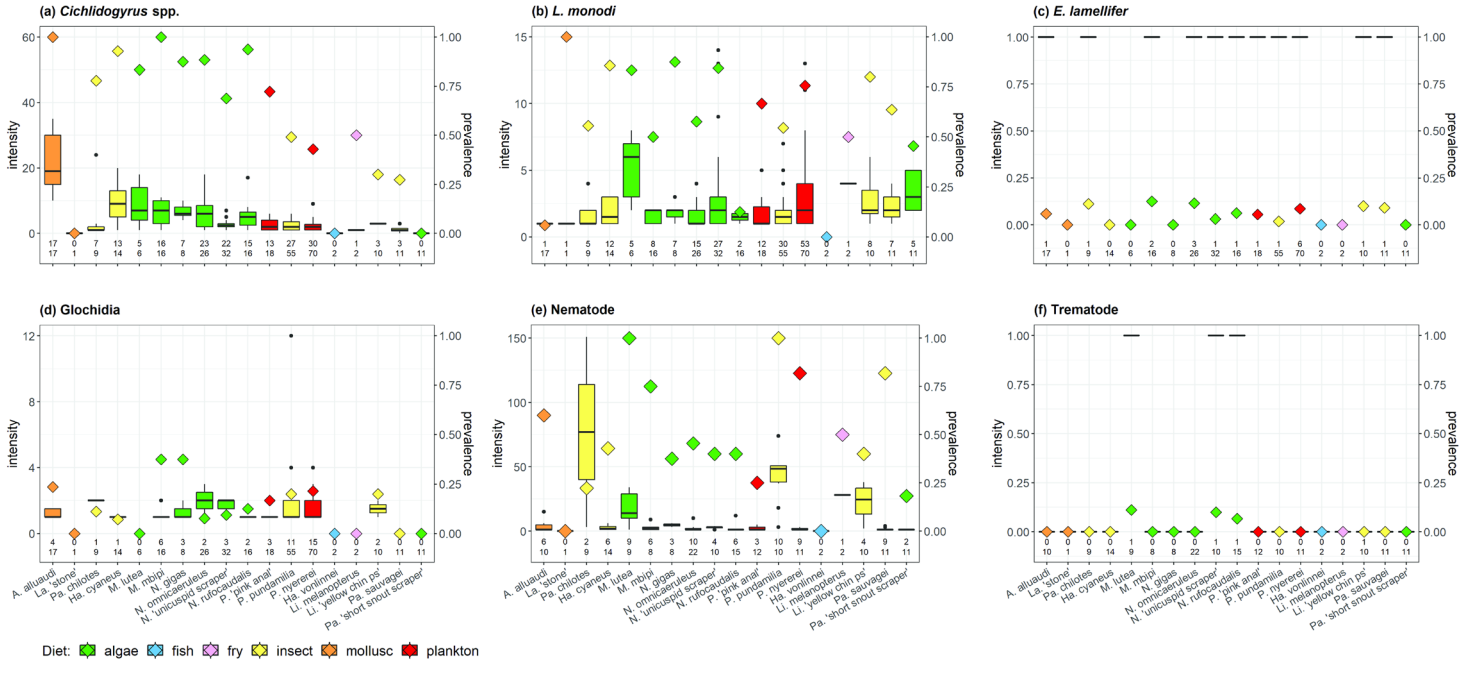

Figure 2.2
Parasite intensity (boxes) and prevalence (diamonds) of cichlid species at Makobe Island in 2014. Colours represent host diet. **(a)** *Cichlidogyrus* spp., **(b)** *Lamproglena monodi*, **(c)** *Ergasilus lamellifer*, **(d)** glochidia, **(e)** nematodes, **(f)** trematodes. Numbers indicate the number of infected fish individuals per species (upper line) and total sample size per species (lower line).

Table 2.2
Parasite infection (% prevalence, mean intensity, mean abundance, abundance range) of cichlid fish at Makobe, Kissenda and Sweya locations in 2014.

Host species	*Cichlidogyrus* spp.				*Lamproglena monodi*				*Ergasilus lamellifer*			
	%	int	abundance		%	int	abundance		%	int	abundance	
Makobe												
A. alluaudi	100.0	20.3	20.3	(2-59)	18.5	1.8	0.3	(0-3)	7.4	1.0	0.1	(0-1)
Ha. serranus	0.0	0.0	0.0	(0-0)	0.0	0.0	0.0	(0-0)	0.0	0.0	0.0	(0-0)
La. sp. 'stone'	53.3	1.3	0.7	(0-2)	53.3	2.3	1.2	(0-7)	0.0	0.0	0.0	(0-0)
Li. melanopterus	70.0	1.6	1.1	(0-3)	40.0	3.8	1.5	(0-5)	0.0	0.0	0.0	(0-0)
Li. sp. 'yellow chin pseudonigricans'	30.0	3.0	0.9	(0-3)	80.0	2.8	2.2	(0-6)	10.0	1.0	0.1	(0-1)
M. lutea	80.0	6.0	5.1	(0-18)	85.0	4.8	4.3	(0-21)	5.0	1.0	0.1	(0-1)
M. mbipi	90.6	6.0	5.8	(0-16)	50.0	1.8	0.9	(0-3)	6.3	1.0	0.1	(0-1)
N. gigas	90.5	6.9	6.2	(0-17)	90.5	2.1	1.9	(0-5)	0.0		0.0	(0-0)
N. omnicaeruleus	88.6	6.0	5.3	(0-18)	54.3	1.7	0.9	(0-4)	8.6	1.0	0.1	(0-1)
N. rufocaudalis	96.0	4.4	4.2	(0-17)	20.0	2.0	0.4	(0-3)	8.0	1.0	0.1	(0-1)
N. sp. 'unicuspid scraper'	67.5	2.6	1.7	(0-7)	82.5	3.3	2.7	(0-14)	10.0	1.0	0.1	(0-1)
P. nyererei	49.4	2.1	1.1	(0-9)	76.5	3.0	2.3	(0-13)	11.1	1.1	0.1	(0-2)
P. sp. 'pink anal'	57.1	2.6	1.5	(0-6)	60.7	1.6	1.0	(0-5)	3.6	1.0	0.0	(0-1)
P. pundamilia	44.6	2.5	1.1	(0-6)	52.3	1.9	1.0	(0-7)	1.5	1.0	0.0	(0-1)
Pa. chilotes	60.0	3.4	2.1	(0-24)	45.0	2.3	1.1	(0-6)	30.0	1.3	0.4	(0-2)
Pa. cyaneus	95.7	7.6	7.3	(0-20)	87.0	2.6	2.3	(0-7)	8.7	1.0	0.1	(0-1)
Pa. sauvagei	13.6	1.7	0.2	(0-3)	68.2	2.9	2.0	(0-9)	9.1	1.0	0.1	(0-1)
Pa. sp 'short snout scraper'	0.0	0.0	0.0	(0-0)	60.0	6.4	3.9	(0-16)	15.0	2.3	0.4	(0-4)
Sweya												
A. alluaudi	66.7	9.0	6.0	(0-33)	0.0	0.0	0.0	(0-0)	0.0	0.0	0.0	(0-0)
Ps. multicolor	25.0	2.4	0.6	(0-5)	0.0	0.0	0.0	(0-0)	5.0	1.0	0.1	(0-1)
Kissenda												
P. sp. 'nyererei-like'	81.0	4.3	3.5	(0-25)	42.9	1.9	0.8	(0-5)	52.4	1.8	0.9	(0-4)
P. sp. 'pundamilia-like'	80.5	5.3	4.3	(0-17)	43.9	1.7	0.8	(0-4)	39.0	1.7	0.7	(0-4)
Pt. xenognathus	60.0	3.5	2.1	(0-9)	50.0	1.6	0.8	(0-4)	70.0	3.0	2.1	(0-7)

Table 2.2 (continued)

Host species	Glochidia				Nematodes				Trematodes			
	%	int	abundance		%	int	abundance		%	int	abundance	
Makobe												
A. alluaudi	25.9	2.3	0.6	(0-5)	60.0	4.2	2.5	(0-15)	0.0	-	0.0	(0-0)
Ha. serranus	0.0	0.0	0.0	(0-0)	0.0	-	0.0	(0-0)	0.0	-	0.0	(0-0)
La. sp. 'stone'	20.0	1.3	0.3	(0-2)	0.0	-	0.0	(0-0)	0.0	-	0.0	(0-0)
Li. melanopterus	0.0	0.0	0.0	(0-0)	0.0	-	14.0	(0-28)	0.0	-	0.0	(0-0)
Li. sp. 'yellow chin pseudonigricans'	20.0	1.5	0.3	(0-2)	30.0	19.0	8.9	(0-38)	0.0	-	0.0	(0-0)
M. lutea	10.0	1.5	0.2	(0-2)	100.0	17.7	17.7	(1-34)	11.1	1.0	0.1	(0-1)
M. mbipi	28.1	1.8	0.5	(0-4)	62.5	3.4	2.3	(0-9)	0.0	-	0.0	(0-0)
N. gigas	19.1	1.3	0.2	(0-2)	37.5	4.7	1.8	(0-6)	0.0	-	0.0	(0-0)
N. omnicaeruleus	5.7	2.0	0.1	(0-3)	27.3	3.0	1.1	(0-10)	0.0	-	0.0	(0-0)
N. rufocaudalis	8.0	1.0	0.1	(0-1)	33.3	3.2	1.1	(0-12)	6.7	1.0	0.1	(0-1)
N. sp. 'unicuspid scraper'	10.0	1.5	0.2	(0-2)	40.0	2.8	1.1	(0-4)	10.0	1.0	0.1	(0-1)
P. nyererei	22.2	2.0	0.5	(0-8)	63.6	1.7	1.4	(0-3)	0.0	-	0.0	(0-0)
P. sp. 'pink anal'	10.7	1.0	0.1	(0-1)	16.7	3.0	0.6	(0-5)	0.0	-	0.0	(0-0)
P. pundamilia	20.0	4.2	0.9	(0-26)	80.0	58.6	52.3	(3-152)	0.0	-	0.0	(0-0)
Pa. chilotes	10.0	2.5	0.3	(0-3)	11.1	3.0	17.1	(0-151)	0.0	-	0.0	(0-0)
Pa. cyaneus	4.4	1.0	0.0	(0-1)	42.9	2.7	1.1	(0-6)	0.0	-	0.0	(0-0)
Pa. sauvagei	0.0	0.0	0.0	(0-0)	72.7	1.6	1.3	(0-4)	0.0	-	0.0	(0-0)
Pa. sp 'short snout scraper'	0.0	0.0	0.0	(0-0)	18.2	1.0	0.2	(0-1)	0.0	-	0.0	(0-0)
Sweya												
A. alluaudi	66.7	17.0	11.3	(0-37)								
Ps. multicolor	10.0	7.0	0.7	(0-13)	27.3	4.7	1.3	(0-10)	0.0	-	0.0	(0-0)
Kissenda												
P. sp. 'nyererei-like'	50.0	7.0	3.5	(0-20)	20.0	1.0	0.2	(0-1)	0.0	-	0.0	(0-0)
P. sp. 'pundamilia-like'	46.3	11.3	5.2	(0-44)	44.4	1.0	0.6	(0-1)	11.1	1.0	0.1	(0-1)
Pt. xenognathus	90.0	16.0	14.4	(0-83)	0.0	-	0.0	(0-0)	0.0	-	0.0	(0-0)

2.3. RESULTS

We observed five ectoparasite taxa and two endoparasite taxa (**Table 2.2**; not considering species diversity of *Cichlidogyrus*). The ectoparasites were: *Cichlidogyrus* spp. (Monogenea: Dactylogyridea), *Gyrodactylus sturmbaueri* (Monogenea: Gyrodactylidea), *Lamproglena monodi* (Copepoda: Cyclopoida), *Ergasilus lamellifer* (Copepoda: Poecilostomatoida) and glochidia mussel larvae (Bivalvia: Unionoidea). Among endoparasites we found nematodes and trematodes.

Trematodes, *E. lamellifer* and glochidia were rarely observed. Only three individuals (from three different species) were infected by trematodes; therefore, we did not perform statistical analyses on these. Representatives of *Cichlidogyrus* and *L. monodi* were common, with prevalence generally higher than 50%. *Gyrodactylus sturmbaueri* was encountered only once (in *Pt. xenognathus* from Kissenda Island). The latter parasite was originally described from *Simochromis diagramma*, a tropheine cichlid from Lake Tanganyika (Vanhove et al., 2011) and was also observed in the haplochromine *Pseudocrenilabrus philander* in Zimbabwe and South Africa (Zahradníčková et al., 2016). The current study is hence the first report of this monogenean species in Lake Victoria.

At Makobe, within radiation members, ectoparasites were more prevalent than endoparasites (84.45% of fish infected with ectoparasites and 48.85% with endoparasites, LR_1=41.56, p<0.0001). Individuals infected by endoparasites tended to have those in larger numbers than ectoparasites, that were usually present in low numbers (mean intensity 11.77±2.73 endoparasites and 7.03±0.72 ectoparasites, LR_1=83.34, p<0.0001). Individuals infected by endoparasites carried more ectoparasites than individuals without endoparasites (7.03±0.72 vs. 4.25±0.51, LR_1=9.17, p=0.002). Also when considering both lineages, radiation members and *A. alluaudi*, prevalence and intensity of endoparasites were higher than those of ectoparasites (prevalence: 85.3% ectoparasites, 49.2% endoparasites, LR_1=46.27, p<0.0001; mean intensity 11.30±2.56 endoparasites and 8.89±1.12 ectoparasites, LR_1=21.26, p<0.0001; **Fig. 2.2**).

2.3.1. Divergent parasite infection across host species

Within the radiation, host species were infected by different parasite communities (ANOSIM on zero-adjusted Bray-Curtis distances R=0.3675, p<0.0001): each species differed in its infection profile from at least five other species and on average from 11 other species (out of 16; **Table 2.3**). Including *A. alluaudi* did not change this pattern, but the parasite community composition of this non-radiating lineage differed from every radiation member (**Table 2.3**). The differences in parasite infection profiles were largely driven by the numbers of parasites of each taxon, rather than by the presence or absence of parasite taxa. Indeed, the same five parasite

taxa were shared by all host species, as illustrated by the few differences in Jaccard indices within the radiation (**Table S2.1a**). To exclude possible effects of uneven sample sizes between host species, we repeated community analysis on host species represented by at least 10 individuals and we performed ectoparasite community analysis on host species from both years. These analyses confirmed the aforementioned patterns (**Tables S2.1b** and **S2.1c**, **S2.4**).

Considering each parasite taxon separately, we found that host species had significantly heterogeneous prevalence and intensity of *Cichlidogyrus* spp., *L. monodi* and nematodes (**Table 2.4**). The prevalence of glochidia tended to differ among host species as well. We found the same pattern of infection differences among host species when including *A. alluaudi* (**Table S2.2a**) and also when accounting for fish standard length (**Table S2.3**). Infected *A. alluaudi* had a significantly higher intensity of *Cichlidogyrus* spp. than all other infected host species (mean intensity 23.23±2.86 vs. 0.45±0.28 - 8.43±1.53, all $p<0.001$). As above, we repeated this analysis on the subset of host species represented by at least 10 individuals. These confirmed the aforementioned patterns, with the exception of *L. monodi* intensity that no longer differed between host species (**Tables S2.2b** and **S2.2c**).

2.3.2. Water depth and diet do not fully explain infection variation

Since haplochromine species occupy different water depth ranges, we investigated if parasite infection covaried with the typical water depth range of each species. Variation in parasite community among radiation members inhabiting Makobe was best explained by host species (15.39%, PERMANOVA $p=0.0001$, $F_{16}=0.269$), rather than diet (2.84%) or water depth (5.30% for 3 m ranges). The contribution of water depth increased with higher-resolution depth categorization (10 m 1.22%, 5 m 3.68%, 3 m 5.30%, 2 m 7.79%, 1 m 9.49%). However, the species contribution was dominant regardless of the depth bin chosen. Including *A. alluaudi* gave similar results (species 18.08%, diet 3.84%, 3-m depth range 4.80%).

A similar pattern was observed for individual parasite taxa: variation in prevalence of *Cichlidogyrus* spp., *L. monodi* and nematodes was best explained by host species, rather than individual capture depth and/or diet (**Table 2.4**). Intensities of *Cichlidogyrus*, *L. monodi* and nematodes were explained by both host species and water depth. Fish individuals from deeper waters had more *L. monodi* and fewer *Cichlidogyrus* and nematodes (**Table 2.4**). However, the effect of depth on the intensities of *Cichlidogyrus* and nematodes differed among host species (follow-up analysis revealed significant species by depth interactions; *Cichlidogyrus*: $LRT_{10}=53.99$, $p<0.0001$; nematodes: $LRT_7=122.57$, $p<0.0001$). Variation in *E. lamellifer* and glochidia (both in terms of prevalence and intensity) was not significantly associated with host species identity, nor with ecological factors (water depth, diet) – at species nor at individual level. Including *A. alluaudi* gave similar results (**Table S2.2a**), as well as including host standard length in the analyses (**Table S2.3**).

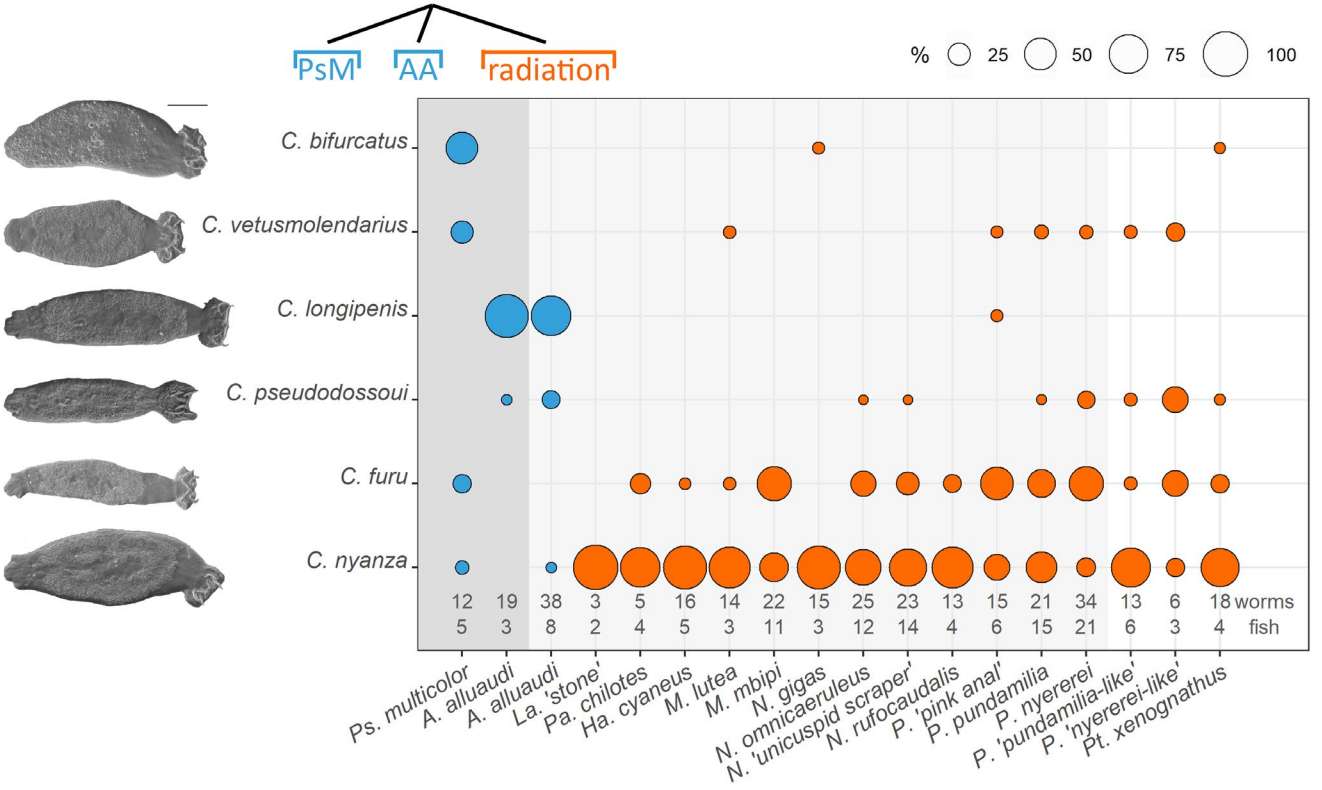

Figure 2.3
Species of *Cichlidogyrus* (micrographs on the left, scale bar 100 µm) infecting cichlid species at Sweya (dark grey background), Makobe Island (light grey background) and Kissenda Island (white background). Infection profiles did not differ among species of the radiation (orange), except for seven (out of 105) comparisons. Infection profiles differed among host lineages, as highlighted by the simplified host phylogeny on top right (PsM *Ps. multicolor*, AA *A. alluaudi*).

Table 2.3
Differences in parasite community (not considering *Cichlidogyrus* species diversity) between cichlid host species at Makobe Island in 2014. Parasite community composition of *Astatoreochromis alluaudi* (non-radiating lineage) differed from all radiation members. Within the radiation (separate analysis), each host species differed from at least five other species in parasite community. Differences are expressed as R values, derived from ANOSIM pairwise comparisons (Benjamini-Hochberg correction) based on zero-adjusted Bray-Curtis distances of parasite abundance, 9999 permutations.

	A. alluaudi	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*	*M. mbipi*	*N. gigas*	*N. omnicaeruleus*
	non-radiating	*radiation*					
Pa. chilotes	**0.782 *** **						
Pa. cyaneus	**0.290 ** **	**0.490 ** **					
M. lutea	**0.861 *** **	**0.604 * **	**0.757 *** **				
M. mbipi	**0.476 ** **	**0.305 * **	0.044	**0.793 ** **			
N. gigas	**0.617 *** **	**0.405 ** **	-0.025	**0.810 ** **	0.009		
N. omnicaeruleus	**0.294 ** **	**0.330 * **	-0.024	**0.663 *** **	-0.077	-0.059	
N. sp. 'unicuspid scraper'	**0.981 *** **	0.060	**0.419 ** **	**0.92 *** **	**0.403 ** **	**0.392 ** **	**0.333 ** **
N. rufocaudalis	**0.592 *** **	**0.414 * **	**0.179 * **	**0.851 *** **	0.086	0.153	0.072
P. sp. ' pink anal'	**0.894 *** **	-0.010	**0.378 ** **	**0.905 *** **	**0.324 * **	**0.325 ** **	**0.310 ** **
P. pundamilia	**0.915 *** **	**0.661 ** **	**0.917 *** **	**0.248 * **	**0.822 *** **	**0.846 *** **	**0.868 *** **
P. nyererei	**0.970 *** **	0.217 .	**0.444 *** **	**0.921 *** **	**0.402 ** **	**0.454 ** **	**0.372 ** **
Ha. vonlinnei	**1.000 * **	-0.052	**0.867 * **	1.000 .	**0.806 * **	**0.987 * **	**0.790 * **
Li. melanopterus	**0.937 * **	0.094	**0.763 * **	0.365	**0.849 * **	0.735 .	**0.741 * **
Li. sp. 'yellow chin pseudonigricans'	**0.742 *** **	-0.019	**0.438 *** **	0.215 .	**0.268 * **	**0.201 * **	**0.343 ** **
Pa. sauvagei	**0.989 *** **	**0.264 * **	**0.596 *** **	**0.928 *** **	**0.565 ** **	**0.619 *** **	**0.537 *** **
Pa. sp. 'short snout scraper'	**1.000 *** **	**0.272 * **	**0.73 *** **	**0.941 ** **	**0.804 *** **	**0.785 *** **	**0.724 *** **

Table 2.3 (continued)

N. sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. ' pink anal'	*P. pundamilia*	*P. nyererei*	*Ha. vonlinnei*	*Li. melanopterus*	*Li.* sp. 'yellow chin pseudonigricans'	*Pa. sauvagei*
0.467 * **								
-0.084	**0.364 ** **							
0.871 * **	**0.904 *** **	**0.901 *** **						
-0.019	**0.496 *** **	0.056	**0.862 *** **					
**0.472 * **	**0.669 * **	0.107	**0.944 * **	**0.755 * **				
0.523 .	**0.896 * **	0.496 .	0.422	**0.604 * **	0.000			
0.060	**0.494 *** **	0.092	**0.475 ** **	**0.175 * **	-0.007	-0.029		
0.059	**0.602 *** **	0.066	**0.865 *** **	-0.041	0.346 .	**0.586 * **	**0.158 * **	
**0.239 * **	**0.74 *** **	0.118 .	**0.938 *** **	**0.350 ** **	-0.177	0.523 .	**0.152 * **	0.168 .

Table 2.4
Variation in prevalence and intensity of parasites (pooling *Cichlidogyrus* species) among host species of the radiation at Makobe Island, in 2014. The minimum adequate model (in bold) was established by stepwise removal of nonsignificant variables (in grey), and the contribution of each fixed effect was assessed through LRT. Model fits were also compared through AIC.

factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
***Cichlidogyrus* spp. prevalence**						***Cichlidogyrus* spp. intensity**					
1					414.57	1					1170.92
species	**16**	**97.58**	**<0.001**	***	**348.99**	species	13	213.57	<0.001	***	983.35
species	16	96.15	<0.001	***	349.68	**species**	**13**	**139.14**	**<0.001**	***	**977.23**
depth	1	1.31	0.252			**depth**	**1**	**8.13**	**0.004**	**	
depth	1	2.75	0.097		413.82	depth	1	82.56	<0.001	***	1090.37
depth	1	0.37	0.545		400.45	depth	1	43.09	<0.001	***	1066.25
diet	5	23.37	<0.001	***		diet	3	30.12	<0.001	***	
diet	5	25.76	<0.001	***	398.82	diet	3	69.59	<0.001	***	1107.33
***Lamproglena monodi* prevalence**						***Lamproglena monodi* intensity**					
1					401.42	1					793.29
species	**16**	**48.06**	**<0.001**	***	**385.36**	species	15	46.10	<0.001	***	777.19
species	16	40.13	0.001	***	387.24	**species**	**15**	**38.12**	**0.001**	***	**769.77**
depth	1	0.12	0.735			**depth**	**1**	**9.42**	**0.002**	**	
depth	1	8.05	0.005	**	395.37	depth	1	17.40	<0.001	***	777.88
depth	1	5.88	0.015	*	397.65	depth	1	13.75	<0.001	***	779.54
diet	5	7.72	0.172			diet	4	6.34	0.175		
diet	5	9.88	0.079	·	401.53	diet	4	10.00	0.040	*	791.29
***Ergasilus lamellifer* prevalence**						***Ergasilus lamellifer* intensity**					
1					**64.35**	**1**					**38.00**
species	16	11.85	0.754		83.50	species	9	0.00	1.000		56.00
species	16	11.11	0.803		84.90	species	9	0.00	1.000		58.00
depth	1	0.15	0.699			depth	1	0.00	1.000		
depth	1	0.89	0.346		66.36	depth	1	0.00	1.000		40.00
depth	1	0.40	0.526		75.63	depth	1	0.00	1.000		44.00
diet	5	1.43	0.922			diet	2	0.00	1.000		
diet	5	1.91	0.861		73.71	diet	2	0.00	1.000		42.00
Glochidia prevalence						**Glochidia intensity**					
1					**271.56**	**1**					**159.52**
species	16	24.24	0.084	·	279.32	species	10	7.63	0.665		171.89
species	16	25.46	0.062	·	280.09	species	10	7.15	0.711		173.08
depth	1	1.23	0.268			depth	1	0.81	0.367		
depth	1	0.00	0.988		273.56	depth	1	1.29	0.256		160.23
depth	1	0.19	0.667		279.98	depth	1	0.56	0.454		161.11
diet	5	3.58	0.611			diet	2	3.12	0.210		
diet	5	3.40	0.639		278.16	diet	2	3.85	0.146		159.67
Nematodes prevalence						**Nematodes intensity**					
1					230.68	1					2698.37
species	**16**	**55.46**	**<0.001**	***	**207.23**	species	14	1790.90	<0.001	***	935.43
species	16	49.35	<0.001	***	208.27	**species**	**14**	**1495.37**	**<0.001**	***	**853.92**
depth	1	0.96	0.328			**depth**	**1**	**83.51**	**<0.001**	***	
depth	1	7.06	0.008	**	225.62	depth	1	379.07	<0.001	***	2321.29
depth	1	7.78	0.005	**	229.46	depth	1	410.58	<0.001	***	1620.64
diet	5	6.16	0.291			diet	3	706.65	<0.001	***	
diet	5	5.45	0.364		235.24	diet	3	675.14	<0.001	***	2029.23

2.3.3. Temporal consistency in infection

Ectoparasite community composition did not differ between the two sampling years (R=0.001, p=0.423; note that endoparasites were not screened in 2010). Temporal fluctuations in the abundance of parasites were observed for some parasite taxa but not others (**Table S2.5**). Overall, prevalence was similar in both sampling years for *Cichlidogyrus* (LRT_1=0.03, p=0.861), *L. monodi* (LRT_1=0.43, p=0.551) and glochidia (LRT_1=1.28, p=0.256). Prevalence of *E. lamellifer* was higher in 2010 (LRT_1=7.86, p=0.005). Infection intensity was lower in 2014 for *L. monodi* (LRT_1=11.56, df=1, p=0.001) and glochidia (LRT_1=14.51, p<0.0001), but similar for *Cichlidogyrus* (LRT_1=1.45, df=1, p=0.227) and *E. lamellifer* (LRT_1=0.37, df=1, p=0.541).

Despite temporal fluctuations in some parasite taxa, differences in infection profile between host species were consistent over time (**Table S2.5**). Most importantly, variation among radiation members in both prevalence and intensity of the two most common parasites, *Cichlidogyrus* and *L. monodi*, were positively correlated between 2010 and 2014 (**Fig. 2.4**, **Fig. S2.5**). Interspecific variation in *Cichlidogyrus* prevalence and in glochidia intensity differed between years. Including *A. alluaudi* gave a similar pattern (**Table S2.5b**).

We focused on several pairs of closely related host species (following Seehausen, 1996b; Magalhaes et al., 2012; Keller et al., 2013; Wagner et al., 2013; Brawand et al., 2014) to assess temporal consistency of parasite-mediated divergent selection within those pairs. If parasite-mediated divergent selection contributes to speciation, its signature should be especially visible in species pairs that are in the process of evolving reproductive isolation. The direction of the infection difference between sister species depended on the ectoparasite taxon and the host pair considered, but in general the direction was maintained over time (visual inspection of **Fig. 2.4**, **Fig. S2.5**; endoparasites were not assessed in 2010). We excluded cases in which prevalence or mean intensity was identical for the two species within a pair in one or both years (respectively, 3 and 4 out of 20 comparisons). Prevalence of glochidia was temporally consistent among all sister pairs; prevalence of *Cichlidogyrus* and *L. monodi* were consistent among most pairs (3 out of 4, 3 out of 5 respectively). Sister species differences in prevalence of *E. lamellifer* were maintained in both years only in the *P. pundamilia* – *P. nyererei* pair. Intensity of *Cichlidogyrus*, *L. monodi* and glochidia (but not of *E. lamellifer*) were consistent for most sister pairs (3 out of 4; 4 out of 5; 3 out of 4 respectively).

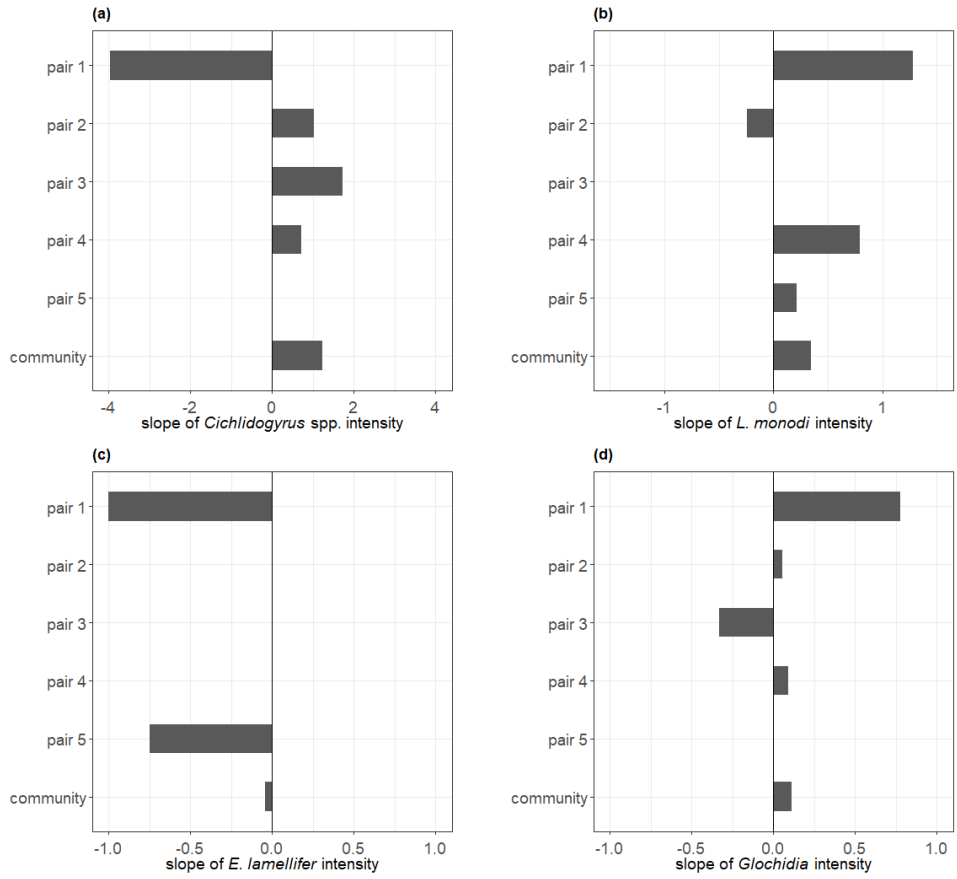

Figure 2.4
Temporal consistency in infection intensity. Correlations between species differences in infection intensity of **(a)** *Cichlidogyrus* spp., **(b)** *Lamproglena monodi*, **(c)** *Ergasilus lamellifer*, **(d)** glochidia between sampling years, for members of the radiation at community wide level and for sister species pairs. After plotting the mean intensity in 2014 against that in 2010 (**Fig. S2.3**), we established the slope of the line connecting the two species within a pair and the slope of the correlation line for all species (for the community-level analysis). A positive correlation slope indicates temporal consistency in infection differences. Intensity of *Cichlidogyrus* spp., *L. monodi* and glochidia were consistent for most sister pairs. Sister species pairs are:
(1) *Mbipia mbipi* – *Mbipia lutea*,
(2) *Mbipia mbipi* – *Pundamilia* sp. 'pink anal',
(3) *Neochromis omnicaeruleus* – *Neochromis* sp. 'unicuspid scraper',
(4) *Pundamilia pundamilia* – *Pundamilia nyererei*,
(5) *Paralabidochromis sauvagei* – *Paralabidochromis* sp. 'short snout scraper'.

2.3.4. Species differences in infection at *Cichlidogyrus* species level

Morphological assessment of *Cichlidogyrus* revealed the presence of six species among the cichlids of the Makobe Island assemblage. These were: *Cichlidogyrus nyanza* n. sp., *C. furu* n. sp. *C. pseudodossoui* n. sp., *C. vetusmolendarius* n. sp., *C. longipenis* and *C. bifurcatus* (taxonomic (re)description in **chapter 6**).

Within the radiation, host species at Makobe harboured similar assemblages of *Cichlidogyrus*, consisting of six species (**Fig. 2.3**). Only two host species (*P. pundamilia*, *P. nyererei*) differed from another radiation member, *N. gigas* (both p=0.036; **Table S2.6a**). This difference was not significant when considering only *Cichlidogyrus* species presence/absence (Jaccard indices, **Table S2.6b**). When excluding host species represented by less than 5 individuals, we observed the same pattern (**Table S2.6c**, **S2.6d**).

To explore differences between species of the radiation and the two species from non-radiating lineages, we examined populations of *A. alluaudi* from Makobe and Sweya, and *Ps. multicolor* from Sweya. Compared to the radiation members, the two populations of *A. alluaudi* had a very different species assemblage of *Cichlidogyrus*, dominated by one species in both populations (*C. longipenis*) that was extremely rare in radiation members (seen only twice, in only one species). At Makobe, *A. alluaudi* differed significantly from almost all radiation members, both considering zero-adjusted Bray-Curtis distances and Jaccard indices (except *La.* sp. 'stone' and *M. lutea*, both p=0.064, probably not reaching statistical significance because of the low sample sizes for these two species; **Table 2.5** and **S2.7b**). The characteristic species community of *Cichlidogyrus* of *A. alluaudi* at Makobe was also found in the Sweya population of this species. Analysis revealed a significant difference in monogenean community composition between allopatric *A. alluaudi*, but this is probably due to their very different sample size (both in terms of fish – 8 Makobe vs. 3 Sweya – and parasite numbers – 38 Makobe vs. 19 Sweya –). The difference disappeared when simulating a larger sample size for Sweya. *Pseudocrenilabrus multicolor* had yet another infection profile, significantly different from the sympatric *A. alluaudi* (zero-adjusted Bray-Curtis p=0.047, Jaccard p=0.035), from *A. alluaudi* inhabiting Makobe (p=0.008, p=0.007) and from several radiation members at Makobe (5 and 3 out of 12 species, respectively). Both diversity indices (zero-adjusted Bray-Curtis and Jaccard) revealed the same pattern, indicating that differences observed in *Cichlidogyrus* communities are due to both numbers and presence/absence of species of *Cichlidogyrus*. When excluding host species represented by less than 5 individuals, we observed the same patterns (**Table S2.7c**, **S2.7d**).

Table 2.5
Differences in *Cichlidogyrus* community between cichlid host species of the radiating and non-radiating lineages at Makobe, Sweya and Kissenda locations. *Cichlidogyrus* community composition of *Astatoreochromis alluaudi* (non-radiating lineage) was similar at Makobe and Sweya but differed from most radiation species. Within the radiation, most species at Makobe had similar *Cichlidogyrus* communities, also similar to radiation members at Kissenda. Differences are expressed as R values, derived from ANOSIM based on zero-adjusted Bray-Curtis distances of species abundances, Benjamini-Hochberg correction, 9999 permutations.

			Ps. multicolor	*A. alluaudi*	*A. alluaudi*	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*
			Sweya		*Makobe*				
			non-radiating lineages			*radiation lineage*			
non-radiating	Sweya	*A. alluaudi*	**0.893 ***						
		A. alluaudi	**0.480 ****	0.393 .					
radiation	Makobe	*La.* sp. 'stone'	0.344	0.834	0.357 .				
		Pa. chilotes	0.367	0.845 .	**0.625 ***	0.125			
		Pa. cyaneus	**0.688 ***	**0.924 ***	**0.775 ***	-0.073	0.344		
		M. lutea	0.604 .	0.972	0.750 .	1.000	1.000	-0.100	
		M. mbipi	**0.427 ***	**0.872 ****	**0.711 ****	0.176	0.025	0.355 .	0.516 .
		N. gigas	0.787 .	0.964 .	**0.759 ***	1.000	1.000 .	-0.036	0.000
		N. omnicaeruleus	**0.362 ***	**0.763 ***	**0.528 ****	-0.235	-0.235	-0.010	0.143
		N. sp. 'unicuspid scraper'	**0.543 ****	**0.875 ****	**0.795 ****	-0.133	-0.199	0.166	**0.535 ***
		N. rufocaudalis	0.601 .	0.919 .	**0.719 ***	0.125	0.427	-0.153	-0.071
		P. sp. 'pink anal'	0.171	0.700	**0.315 ****	-0.195	-0.152	0.132	0.234
		P. pundamilia	**0.560 ***	**0.853 ****	**0.770 ****	-0.297	-0.046	0.073	0.578 .
		P. nyererei	0.292 .	**0.795 ***	**0.523 ****	-0.112	-0.228	**0.154 ***	0.346 .
	Kissenda	*P.* sp. 'pundamilia-like'	0.120	0.811	**0.333 ***	0.125	0.398	0.615 .	0.333
		P. sp. 'nyererei-like'	**0.281 ***	**0.792 ***	**0.546 ****	0.256	0.056	0.440	0.548
		Pt. xenognathus	0.620 .	0.876 .	**0.667 ***	-0.125	0.352	-0.103	-0.417

Table 2.5 (continued)

M. mbipi	*N. gigas*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. 'pink anal'	*P. pundamilia*	*P. nyererei*	*P.* sp. 'pundamilia-like'	*P.* sp. 'nyererei-like'
Makobe								*Kissenda*	
radiation lineage									
0.620 *									
0.013	0.257								
0.082	**0.553 ***	-0.016							
0.250 .	0.222	-0.077	0.111						
0.066	0.375	-0.030	0.126	0.026					
0.276	**0.639 ****	-0.102	-0.003	0.119	0.009				
0.006	**0.493 ****	-0.025	0.001 .	0.046	-0.025	-0.015			
0.211	0.796	0.097	0.330	0.491 .	-0.083	0.491	0.045		
0.022 .	0.781 .	0.129	0.149	0.272	0.098	0.281	0.038 .	-0.003 .	
0.346	0.037	0.025	0.277	-0.111	0.046	0.167	0.222 .	0.315	0.485

The highly similar infection profiles of *Cichlidogyrus* species in *A. alluaudi* from different habitats and locations (Sweya and Makobe) suggests that host species identity determines infection much more than geographic location. To verify this, we also analysed three additional species of the radiation from a third location, Kissenda. At Kissenda, *P.* sp. 'pundamilia-like' and *P.* sp. 'nyererei-like' had infection profiles that were highly similar to that of their counterparts at Makobe, *P. pundamilia* and *P. nyererei* (p=0.614, p=0.547 respectively) despite their substantial geographical distance (23.1 km).

The Makobe sample included only two molluscivore species (*La.* sp. 'stone' and *A. alluaudi*). To assess whether the distinct infection profile of *A. alluaudi* could be explained by its molluscivore diet, we therefore also sampled *Pt. xenognathus* at Kissenda, which is a radiation member (but does not occur at Makobe). The two radiation molluscivores (*Pt. xenognathus* at Kissenda and *La.* sp. 'stone' at Makobe) had similar *Cichlidogyrus* assemblages (p=0.758), that differed from that of *A. alluaudi* at Makobe (p=0.034, **Table 2.5**, **Fig. 2.3**). Thus, molluscivory does not explain the characteristic *Cichlidogyrus* infection profile of *A. alluaudi*. Within the radiation, *Cichlidogyrus* community composition did not significantly differ among the three Kissenda species (all p>0.093) and among them and other radiation members at Makobe (all p>0.051), confirming the modest influence of geographical distance.

2.4. DISCUSSION

We investigated patterns of ecto- and endo-parasite infection in Lake Victoria cichlid fish, to explore potential occurrence of parasite-mediated selection. Consistent with parasite-mediated speciation, we found significant differences between members of the haplochromine radiation in parasite infection levels and parasite communities. These infection differences could not be attributed to host ecology (depth and diet) and were largely consistent over two sampling years. These findings are in line with two prerequisites of parasite-mediated speciation: infection differences between closely related host species, that are temporally consistent. However, at the species level for *Cichlidogyrus*, a common and species-rich genus of monogeneans, we found homogeneous infection profiles within the Lake Victoria radiation, inconsistent with a role of *Cichlidogyrus* species in host speciation. We observed divergent *Cichlidogyrus* infections, that were not due to host ecology nor to geography, only between the radiation cichlids and two distantly related, non-radiating haplochromine lineages. These results suggest that parasite resistance may differ between radiating and non-radiating lineages, but do not support a role of *Cichlidogyrus* in driving divergence within the Lake Victoria haplochromine radiation.

2.4.1. Parasite infection differences among species and the role of ecology

Host species had different parasite infection profiles, as also found by previous studies on the same host assemblage (Maan et al., 2008; Karvonen et al., 2018) and as predicted by the first prerequisite of parasite-mediated speciation (Karvonen & Seehausen, 2012). Significant differences between host species were observed both at the parasite community level and for three out of five individual parasite taxa. Cichlid species in Lake Victoria display different ecological specialisations, inhabiting different water depth ranges and specialising on different dietary resources (Seehausen, 1996b; Bouton et al., 1997; Seehausen & Bouton, 1997). This likely translates into differences in parasite exposure. Intensity of some parasites (*Cichlidogyrus* spp., *L. monodi* and nematodes) were indeed associated with water depth, but water depth and diet did not fully explain the variation in infection profile between host species.

Hosts from deeper waters had more *L. monodi* and fewer *Cichlidogyrus* and nematodes, consistent with differences in parasite ecology and thereby exposure to those parasites. *Lamroglena monodi* is a fully limnetic copepod with a direct life cycle and its infective stage can survive a few days without a host (Paperna, 1996). These characteristics may lead to high dispersal and allow *L. monodi* to infect deep-water dwelling fish. Representatives of *Cichlidogyrus* have a direct life cycle: eggs are released by adults from the fish host and the infective free-swimming larvae have only a few hours to find a suitable host (Paperna, 1996). Higher host densities in shallow waters may provide favourable conditions for *Cichlidogyrus* transmission. Nematodes were found in the abdominal cavity only, indicating that cichlids are intermediate hosts (Yanong, 2017). Most nematodes have an indirect life cycle with birds as final hosts, that release eggs through faeces. Thus, nematode transmission is highest close to the shoreline, where birds live, and in shallow waters, as discussed below. Some parasites (*E. lamellifer* and glochidia) were not linked to host species, diet or water depth, suggesting that other factors may determine their infection prevalence and intensity, or that *E. lamellifer* and glochidia are generalist parasites that equally infect all sampled radiation members. Many Ergasilids are known to specialise on specific infection sites on fish gills, rather than specific host species (Fryer, 1968; Scholz et al., 2018). Although glochidia are the parasitic larval forms of several bivalve species, they were not more common in molluscivore hosts than in other trophic groups, suggesting that glochidia are not directly ingested trophically.

Endoparasites (dominated by nematodes) showed different prevalences among host species, and variation in intensity across species and water depth ranges, suggesting that they could contribute to divergent selection. In particular, all individuals of two host species (*P. pundamilia* and *M. lutea*) were infected by high numbers of nematodes. Both species live cryptically in very shallow water (1 m) and close to the rocky shore (Seehausen, 1996b), which likely exposes them to nematode eggs released through faeces of piscivorous birds. Similar patterns were observed

in 2003 by Maan et al. (2008), who found that all *P. pundamilia* were infected by nematodes, and with higher intensity than its deeper and more offshore-dwelling sister species *P. nyererei*.

Overall, our results are in line with a previous study on the same host species assemblage (Karvonen et al., 2018). In both that study and ours (sampling years 2010 and 2014), some parasite taxa were related to host depth and diet, but host species identity was always the strongest predictor of infection. The observation that infection divergence between host species could not be explained by ecological factors alone suggests the presence of host species-specific resistance or tolerance, against the parasites that are most important for that particular host species. However, disentangling the contributions of exposure, resistance, tolerance and susceptibility to variation in infection requires experimental manipulation.

2.4.2. Variation in parasite infections between years

For the two most prevalent ectoparasite taxa, *Cichlidogyrus* and *L. monodi*, differences between host species in infection parameters were similar between sampling years. This was true within the radiation but also within sister species pairs: most pairs maintained the direction of the infection difference between them for these two taxa (as well as for glochidia). In an earlier study in one of those species pairs (*Pundamilia*), sampled in 2003, Maan et al. (2008) reported the same direction of infection difference. In the context of rapid evolution, as for the Lake Victoria radiation, even short-term fluctuations in divergent selection may be important for the evolution of reproductive isolation (Siepielski et al., 2009). Therefore, the maintenance of species differences in infection, even over the relatively short time frames studied here (a period of 4 years for most species; a 16-year period for *Pundamilia* sp. when including the 2003 investigation by Maan et al. (2008)) is noteworthy, and suggests that an important prerequisite for divergent selection may be met. However, the potentially rapid turnover of MHC alleles and stochasticity in the direction of parasite-mediated selection (Eizaguirre et al., 2009c; Lenz et al., 2009), shows that longer-term studies are still needed. Also, we did not find consistency for all parasites. For example, *E. lamellifer* did not show temporal constancy for most of the host species pairs. Because of the low prevalence of this parasite, this finding is difficult to interpret.

The observed consistency in the direction of parasite-mediated selection occurred despite variation in its strength, i.e. despite fluctuations between years in overall ectoparasite intensity. Both copepods and glochidia showed lower infection intensity in 2014 than in 2010. This is in line with Maan et al. (2006b), who found that the abundance of parasites varies between years. This variation could result from temporal variation in various ecological factors (e.g. host abundance, water chemistry, climate) and/or from interspecific competition between parasites. For example, Maan et al. (2006b) observed that an increase in the abundance of *L. monodi* coincided with a decrease in *Cichlidogyrus*. We found a similar pattern: the abundance of *Cichlidogyrus* tended to increase from 2010 to 2014, while the abundance of *L. monodi*

decreased. Observations in other fish species have also suggested antagonistic interactions between gill-infecting copepods and monogeneans (Baker et al., 2005).

2.4.3. Host phylogenetic signature of *Cichlidogyrus* infection

Within the radiation, host species differed in the prevalence and intensity of *Cichlidogyrus* infection. However, the species community of *Cichlidogyrus* was similar within the radiation, contrary to our prediction of parasite-driven diversification. This homogeneity in infection among recently arisen host species indicates that *Cichlidogyrus*-species-mediated selection does not contribute to the early stages of speciation.

In contrast to the pattern within the radiation, prevalence and intensity of *Cichlidogyrus* differed significantly between all radiation members and *A. alluaudi* (**Fig. 2.2**). This host species showed a 100% prevalence and harboured high numbers of *Cichlidogyrus* (2.5 times higher than the most heavily infected radiation species, *Pa. cyaneus*). Species identification of *Cichlidogyrus* revealed that this high intensity in *A. alluaudi* was not due to the accumulation of many worm species, but resulted from a high number of individuals from a limited number of species. These findings are partially in contrast with our hypothesis of parasite-mediated selection. *Cichlidogyrus*-mediated divergent selection should result in lower infection intensities in radiation members, which we observed, but also in fewer species per host, and more differentiated species communities among hosts – which we did not observe. Moreover, the other representative from a non-radiating lineage, *Ps. multicolor*, did not exhibit higher *Cichlidogyrus* infection than radiation members (**Table 2.2**). Thus, our findings suggest that while radiating and non-radiating lineages may differ in *Cichlidogyrus* resistance, variation in infection profiles within the radiation do not result from species-specific resistance.

The high intensity of *Cichlidogyrus* in *A. alluaudi* cannot be explained by its molluscivore diet, as two molluscivore radiation members (*La.* sp. 'stone' and *Pt. xenognathus*) had much lower infections. Likewise, the community composition of species of *Cichlidogyrus* was significantly different between *A. alluaudi* and the two molluscivore radiation members (**Fig. 2.3**). The other old and non-radiating lineage, represented by *Ps. multicolor*, harboured a community of *Cichlidogyrus* that differed from radiation members as well as from *A. alluaudi*. The pattern that emerges is that, with a few exceptions, species of *Cichlidogyrus* that infect members of the radiation do not infect old lineages and vice versa. This lineage-specificity occurs even in the presence of many sympatric host species, providing ample opportunity for host switching. Possibly, cross-infection between lineages is hampered by specific co-evolutionary adaptations in the old lineages of *A. alluaudi* and *Ps. multicolor*, which prevents these species of *Cichlidogyrus* from infecting the radiating lineage. Colonisation of other host species, phylogenetically related or co-occurring with the original host, has been observed previously in monogeneans infecting gobies (Huyse & Volckaert, 2005) and cichlids (Mendlová et al., 2012), indicating that parasites

can colonize host species that represent a similar resource without any prior novelty evolution (Agosta & Klemens, 2008). The Lake Victoria radiation may be too recent to represent multiple different resources for parasites, and thus to allow for co-evolutionary differentiation. A similar pattern was observed in closely related cichlids of West African rivers and lakes, which were infected by similar monogenean assemblages (Pariselle et al., 2003). In contrast, the representatives of *Cichlidogyrus* infecting the much older Lake Tanganyika cichlid tribes generally exhibit higher host specificity (Pariselle et al., 2015).

Species of *Cichlidogyrus* that dominate in radiation members were rare in the two non-radiating lineages, and vice versa. This suggests that *Cichlidogyrus* species did not simply sort among cichlid species during the radiation. Instead, ancestral *Cichlidogyrus* may have adapted to the new niche provided by the radiation, and subsequently diversified into the currently observed species - thus specialising on the radiating lineage as a whole, without within-radiation differentiation. Genetic analysis is required to resolve this (as in Vanhove et al., 2015). Such analysis may also reveal genetic variation within *Cichlidogyrus* species, potentially uncovering more differentiated infections within the radiation. Indeed, molecular investigations have already revealed the presence of several cryptic *Cichlidogyrus* species, that are more host-specific than the currently recognized species (e.g. monogeneans Pouyaud et al., 2006; trematodes Jousson et al., 2000; Donald et al., 2004;).

In addition to differences at the level of host lineages, assemblages of *Cichlidogyrus* species may align with host genus. For example, *Pundamilia* spp. (including five species, from two locations) had infection patterns that were more similar to each other than to other radiation members. The same was observed for three species of *Neochromis* (not for *N. gigas*, but sample size was low for this species). These patterns corroborate the phylogenetic signature of *Cichlidogyrus* infections, but require more systematic analysis.

Monogenean intensity differed between sampling sites. At Makobe, *A. alluaudi* harboured a high *Cichlidogyrus* intensity, whereas its allopatric conspecifics at Sweya, as well as the representative of the other old lineage sampled there (*Ps. multicolor*), had low numbers of *Cichlidogyrus*. Abundances of all ectoparasites at Sweya were very low in both host species sampled there, compared to those observed in the radiation members at Makobe. Differences in parasite abundances among sampling sites were also found in Lake Tanganyika haplochromine cichlids (Raeymaekers et al., 2013; Hablützel et al., 2017). The overall lower ectoparasite abundance at Sweya may be explained by habitat conditions. Sweya is a vegetated swampy stream inlet, inhabited by only five fish species, at low abundances. Makobe is a rocky offshore reef inhabited by a large cichlid community with several highly abundant species, and several non-cichlids (Seehausen, 1996b). Low abundance and low diversity of hosts may therefore explain the low numbers of parasites at Sweya. This is in line with Karvonen et al. (2018), who found that within

the Makobe community, host-specific parasite abundance was positively correlated with host-specific population abundance.

Despite differences in overall *Cichlidogyrus* abundance, the community composition of *Cichlidogyrus* in different host lineages was consistent across sampling sites. Allopatric *A. alluaudi* at Makobe and Sweya were infected by identical assemblages (**Fig. 2.3**). The same pattern was observed in four host species from the radiating lineage, sampled at Makobe and Kissenda: two closely related species pairs (*P. nyererei* and *P.* sp. 'nyererei-like', *P. pundamilia* and *P.* sp. 'pundamilia-like') and allopatric species from the same guild (molluscivores *La.* sp. 'stone' and *Pt. xenognathus*) had the same community of species of *Cichlidogyrus* at the two locations. The maintenance of parasite community composition despite geographical separation is consistent with observations in Lake Tanganyika, where allopatric populations of tropheine cichlids harboured the same *Cichlidogyrus* species, while sympatric host species had different infection profiles (Grégoir et al., 2015; Vanhove et al., 2015).

2.5. CONCLUSION

At parasite community level, we found significant differences in infection profiles between host species, that were consistent over time. These findings support parasite-mediated selection in Lake Victoria cichlids. However, the association between host species divergence and parasite infection depended on the parasite taxon considered. At the level of species community of *Cichlidogyrus*, infection profiles were similar within the radiation but different between host lineages. This is not consistent with parasite-mediated diversification within the Lake Victoria radiation. Future genetic analysis of *Cichlidogyrus* species may reveal cryptic parasite diversity between host species within the radiation, that could be congruent with parasite-mediated diversification.

2.6. ACKNOWLEDGEMENTS

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2.7. SUPPLEMENTARY MATERIAL

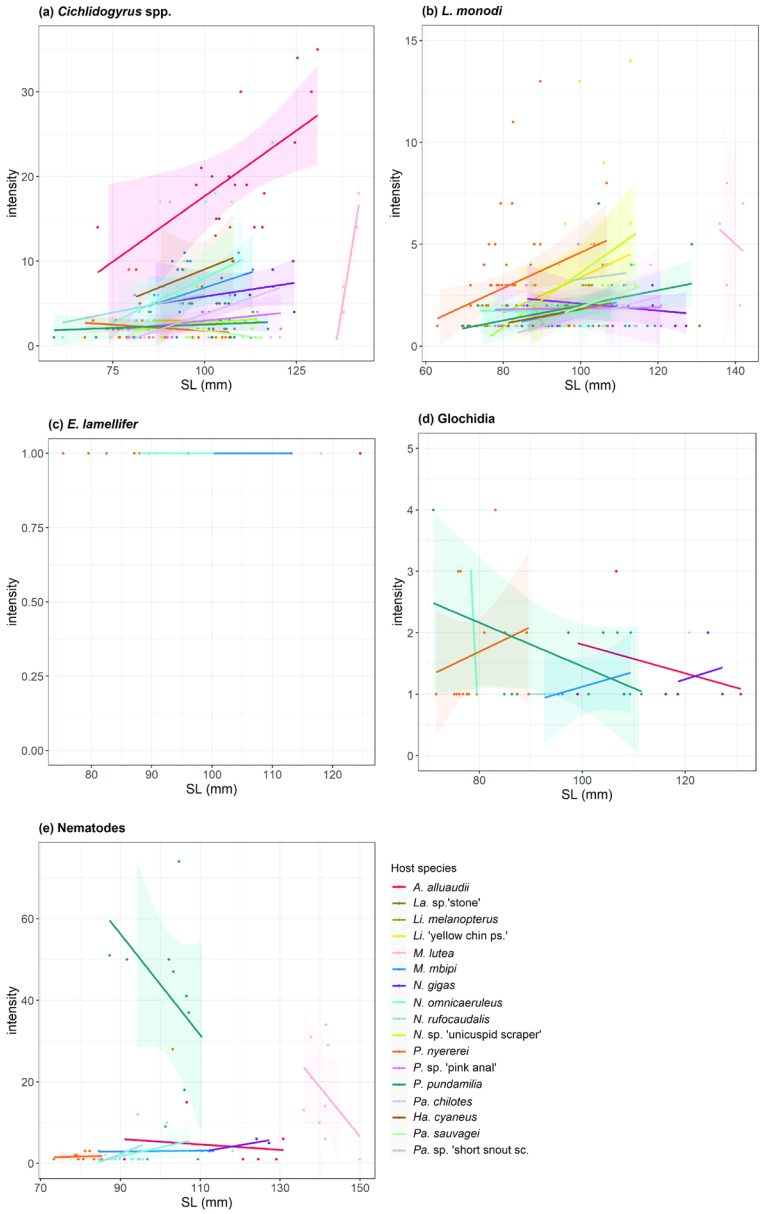

Figure S2.1
Correlations between fish body length (SL, mm) of Makobe cichlids and infection intensity of **(a)** *Cichlidogyrus* spp., **(b)** *L. monodi*, **(c)** *E. lamellifer*, **(d)** glochidia, **(e)** nematodes (data from 2014). The correlation direction varied between host species (*Cichlidogyrus* spp., *L. monodi*, nematode: all $p<0.001$; *E. lamellifer* $p=0.99$; glochidia $p=0.68$).

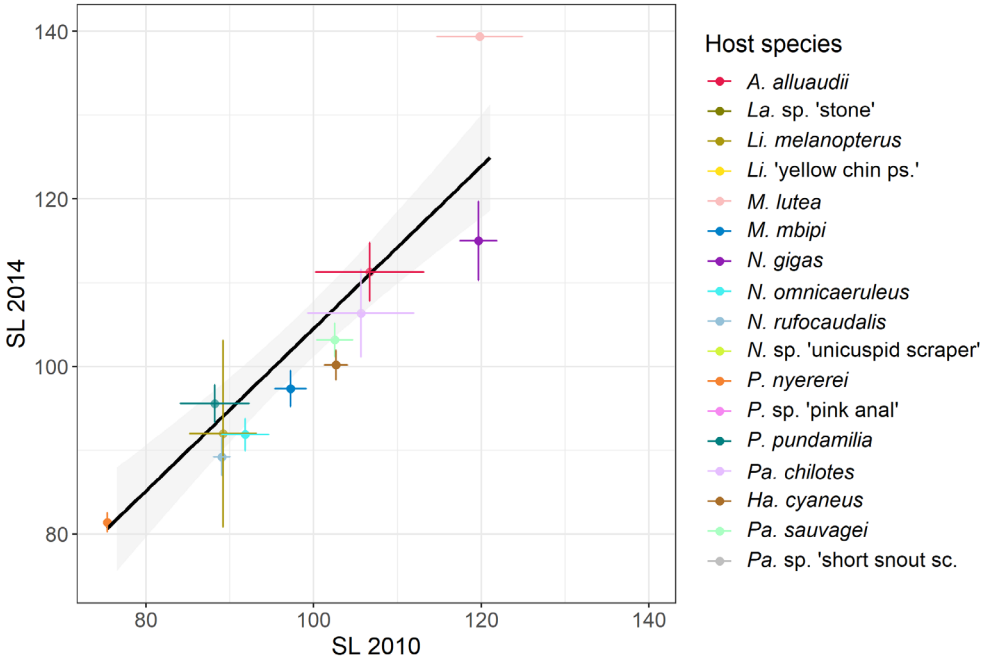

Figure S2.2
Temporal consistency in host species body length. Correlations between average fish body length (SL) between sampling years, for members of the radiation and *Astatoreochromis alluaudi* at Makobe Island.

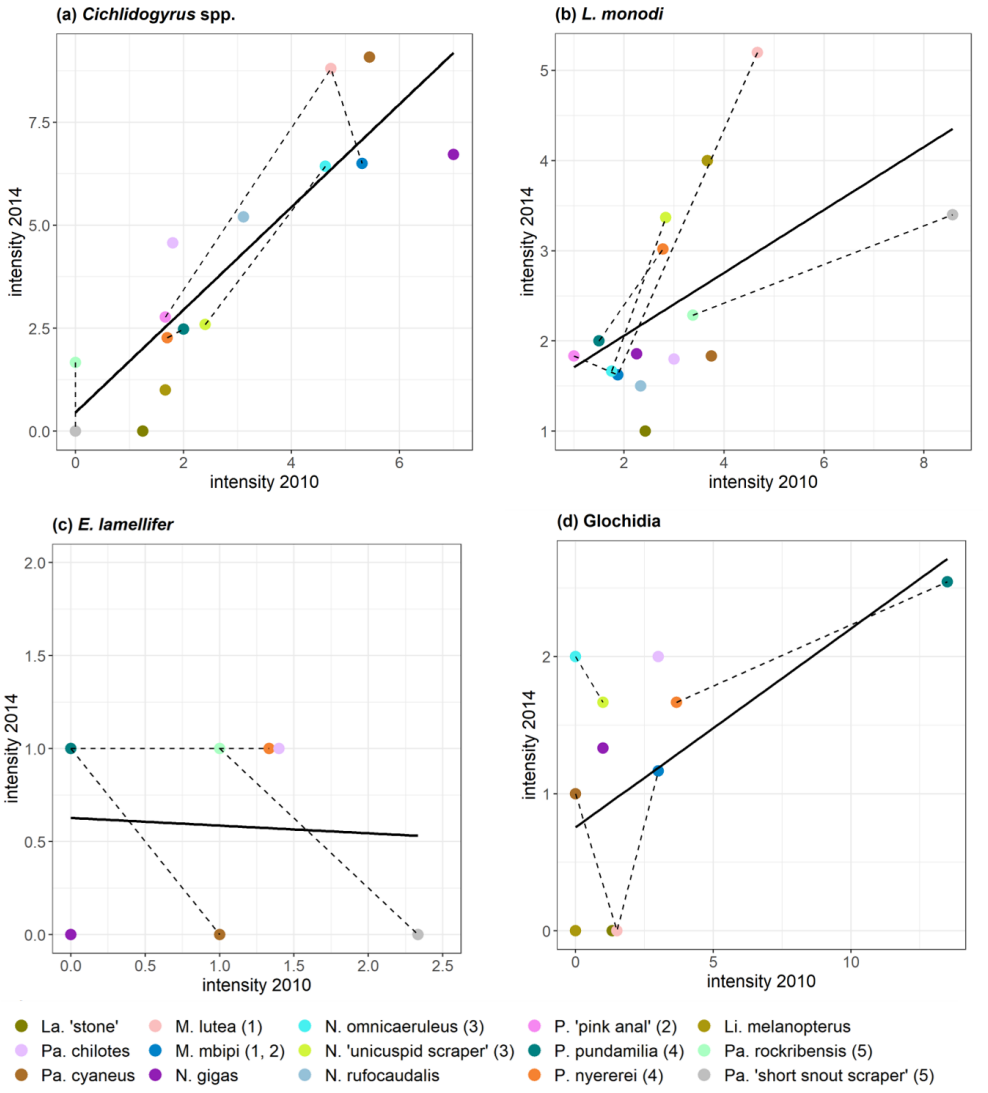

Figure S2.3
Temporal consistency in infection intensity. Correlations between infection intensity of four common macroparasites **(a-d)** between sampling years, for members of the radiation at community wide level (solid line) and for sister species pairs (dashed lines). Sister species pairs are:
(1) *Mbipia mbipi – Mbipia lutea*,
(2) *Mbipia mbipi – Pundamilia* sp. 'pink anal',
(3) *Neochromis omnicaeruleus – Neochromis* sp. 'unicuspid scraper',
(4) *Pundamilia pundamilia – Pundamilia nyererei*,
(5) *Paralabidochromis sauvagei – Paralabidochromis* sp. 'short snout scraper'.

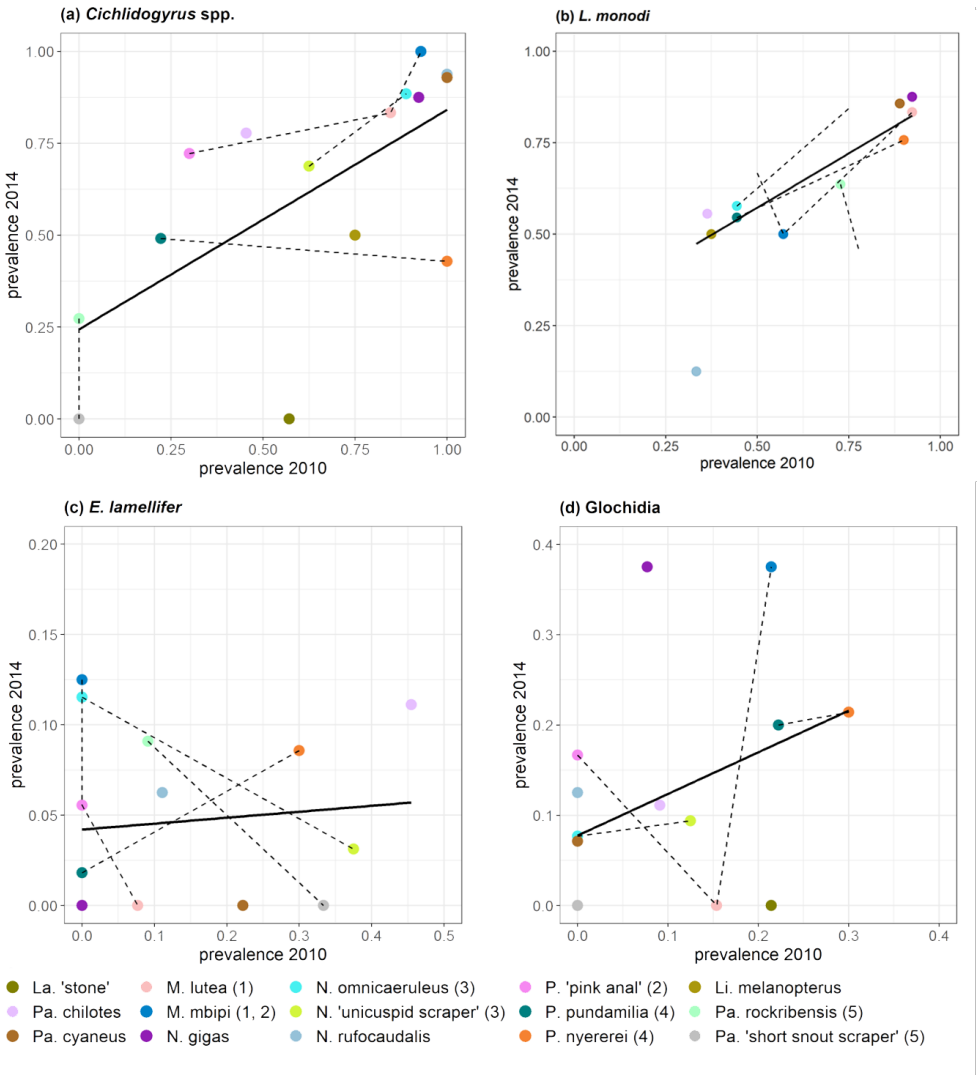

Figure S2.4
Temporal consistency in infection prevalence. Correlations between infection prevalence of four common macroparasites **(a-d)** between sampling years, for members of the radiation at community wide level (solid line) and for sister species pairs (dashed lines). Sister species pairs are:
(1) *Mbipia mbipi* – *Mbipia lutea*,
(2) *Mbipia mbipi* – *Pundamilia* sp. 'pink anal',
(3) *Neochromis omnicaeruleus* – *Neochromis* sp. 'unicuspid scraper',
(4) *Pundamilia pundamilia* – *Pundamilia nyererei*,
(5) *Paralabidochromis sauvagei* – *Paralabidochromis* sp. 'short snout scraper'.

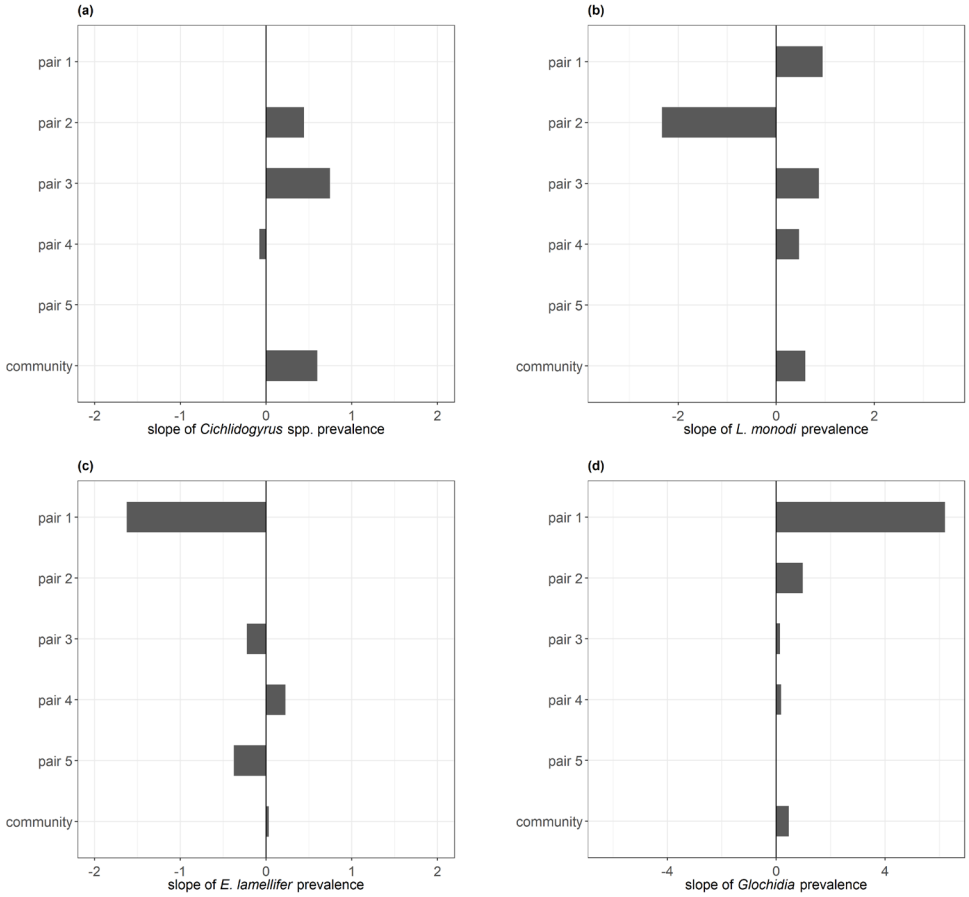

Figure S2.5
Temporal consistency in infection prevalence. Correlations between infection prevalence of **(a)** *Cichlidogyrus* spp., **(b)** *Lamproglena monodi*, **(c)** *Ergasilus lamellifer* and **(d)** glochidia between sampling years, for members of the radiation at community wide level and for sister species pairs. After plotting the prevalence in 2014 against that in 2010 (**Fig. S2.4**), we established the slope of the line connecting the two species within a pair and of the slope of the correlation line for all species (for the community-level analysis). A positive correlation slope indicates temporal consistency in infection differences. Prevalence of *Cichlidogyrus*, *L. monodi* and glochidia were consistent for most sister pairs. Sister species pairs are:
(1) *Mbipia mbipi* – *Mbipia lutea*,
(2) *Mbipia mbipi* – *Pundamilia* sp. 'pink anal',
(3) *Neochromis omnicaeruleus* – *Neochromis* sp. 'unicuspid scraper',
(4) *Pundamilia pundamilia* – *Pundamilia nyererei*,
(5) *Paralabidochromis sauvagei* – *Paralabidochromis* sp. 'short snout scraper'.

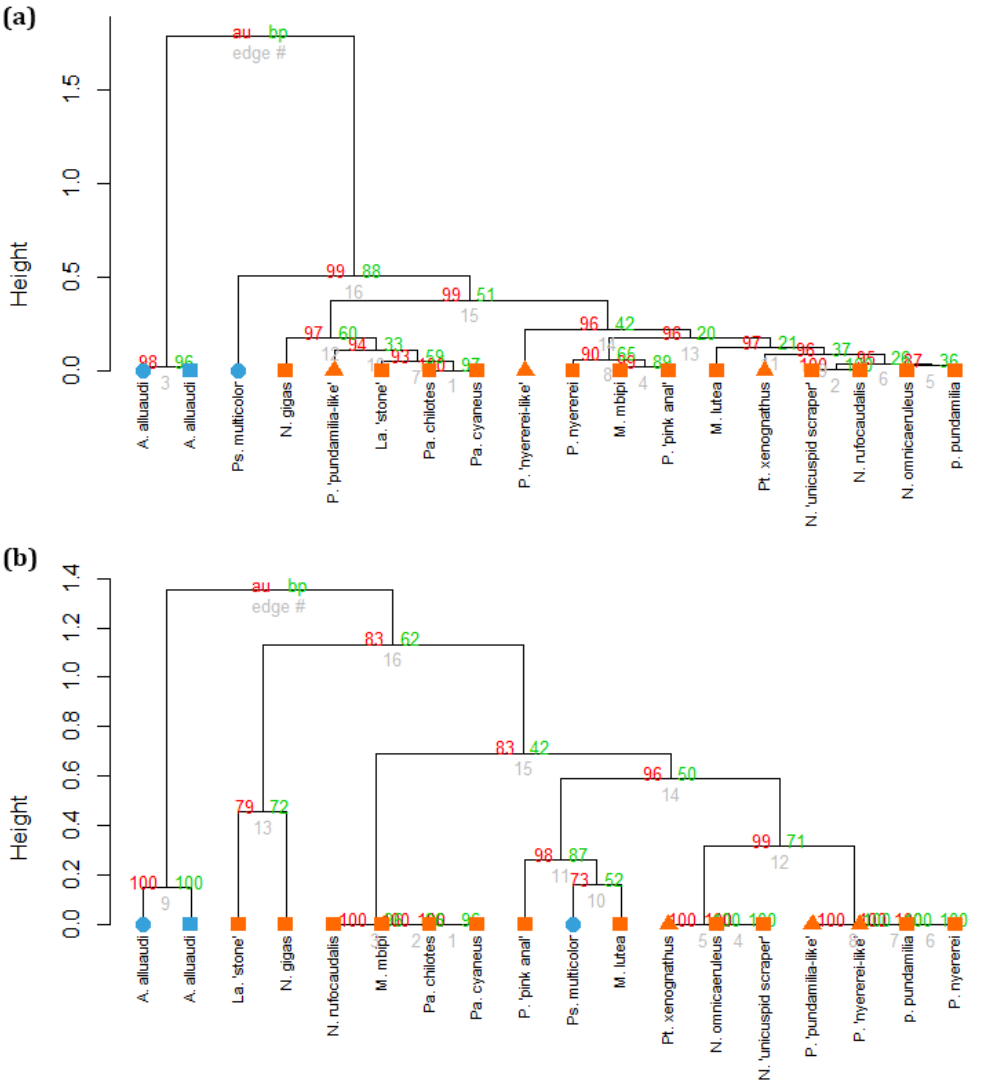

Figure S2.6
Hierarchical clustering dendrograms of *Cichlidogyrus* species communities infecting radiation members (orange) and cichlid species of the non-radiating lineages (*Astatoreochromis alluaudi* and *Pseudocrenilabrus. multicolor*, blue) at Makobe (■), Kissenda (▲) and Sweya (●) locations. Hierarchical clusters calculated on **(a)** zero-adjusted Bray-Curtis distances, **(b)** Jaccard indices, using group average algorithm, 1000 permutations. The *Cichlidogyrus* community of *A. alluaudi* (both Makobe and Sweya locations) constitutes a distinct group, different from radiation members and from the other non-radiating lineage (regardless of the diversity index considered). The *Cichlidogyrus* community of *Ps. multicolor* is distinct from radiation members in the zero-adjusted Bray-Curtis dendrogram, but not in the Jaccard dendrogram.

Table S2.1 (a)
Differences in parasite community (pooling *Cichlidogyrus* species) between cichlid host species at Makobe Island in 2014, expressed as R values based on Jaccard indices, (zero-adjusted Bray-Curtis distances for all host species are given in the main text; **Table 2.3**). Parasite community composition of *Astatoreochromis alluaudi* (non-radiating lineage) differed from most **(a, b)** or all **(c)** radiation members. ANOSIM pairwise comparisons (Benjamini-Hochberg correction), 9999 permutations.

(a)	*A. alluaudi*	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*	*M. mbipi*	*N. gigas*	*N. omnicaeruleus*
	non-radiating	*radiation*					
Pa. chilotes	0.074						
Pa. cyaneus	**0.362 ****	0.209					
M. lutea	0.275 .	0.207	0.067				
M. mbipi	-0.025	0.045	0.144	-0.020			
N. gigas	**0.264 ***	0.089	0.014	0.182	0.055		
N. omnicaeruleus	0.100	0.046	0.016	-0.051	-0.040	0.010	
N. sp. 'unicuspid scraper'	**0.427 ****	0.077	0.056	0.025	0.173 .	0.017	0.076
N. rufocaudalis	-0.040	0.066	**0.271 ****	0.226 .	0.060	**0.213 ***	0.089
P. sp. ' pink anal'	**0.209 ***	-0.097	0.033	-0.050	0.026	-0.061	0.019
P. pundamilia	**0.404 ****	**0.432 ****	**0.42 ****	0.106	**0.246 ***	**0.445 ***	**0.29 ****
P. nyererei	**0.396 ****	0.243 .	0.086	-0.118	0.085	0.133	0.032
Ha. vonlinnei	0.973 .	0.75 .	**0.967 ***	0.938 .	0.966 .	0.966 .	**0.985 ***
Li. melanopterus	0.252	-0.021	0.413	0.490	0.188	0.325	0.282
Li. sp. 'yellow chin pseudonigricans'	**0.383 ****	0.072	**0.256 ***	0.031	0.172 .	0.032	**0.210 ***
Pa. sauvagei	**0.447 ****	**0.300 ***	**0.341 ****	-0.003	**0.224 ***	**0.284 ***	**0.259 ****
Pa. sp. 'short snout scraper'	**0.399 ****	0.075	**0.406 ****	0.026	**0.237 ***	0.125	**0.376 ****

Table S2.1 (continued)

	N. sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. ' pink anal'	*P. pundamilia*	*P. nyererei*	*Ha. vonlinnei*	*Li. melanopterus*	*Li.* sp. 'yellow chin pseudonigricans'	*Pa. sauvagei*
radiation (cont.)									
	0.351 **								
	-0.075	**0.213 ***							
	0.343 **	**0.366 ****	**0.26 ***						
	0.050	**0.345 ****	0.056	0.098					
	0.891 .	**0.906 ***	0.612	**0.978 ***	**0.946 ***				
	0.140	0.305	-0.059	0.335	0.320	0.000			
	-0.019	**0.467 ****	-0.012	**0.316 ****	0.121	0.652	-0.045		
	0.137	**0.475 ****	0.136 .	-0.015	0.028	**0.839 ***	0.115	0.093	
	0.096	**0.574 ****	0.096 .	**0.271 ***	**0.226 ****	0.196	-0.119	0.004	0.128 .

Table S2.1 (b, c)
Differences in parasite community (pooling *Cichlidogyrus* species) between cichlid host species at Makobe Island in 2014, expressed as R values based on **(b)** Jaccard indices, **(c)** zero-adjusted Bray-Curtis distances, considering host species represented by at least 10 individuals (zero-adjusted Bray-Curtis distances for all host species are given in the main text; **Table 2.3**). Parasite community composition of *Astatoreochromis alluaudi* (non-radiating lineage) differed from most (**a**, **b**) or all (**c**) radiation members. ANOSIM pairwise comparisons (Benjamini-Hochberg correction), 9999 permutations.

(b)	*A. alluaudi*	*Pa. cyaneus*	*N. omnicaeruleus*	*N. sp. 'unicuspid scraper'*	*N. rufocaudalis*	*P.* sp. ' pink anal'
	non-radiating	*radiation*				
Pa. cyaneus	**0.362 ****					
N. omnicaeruleus	0.100	0.016				
N. sp. 'unicuspid scraper'	**0.427 ****	0.056	0.076			
N. rufocaudalis	-0.040	**0.271 ****	0.089	**0.351 ****		
P. sp. ' pink anal'	**0.209 ***	0.033	0.019	-0.075	**0.213 ****	
P. pundamilia	**0.404 ****	**0.420 ****	**0.290 ****	**0.343 ****	**0.366 ****	**0.260 ***
P. nyererei	**0.396 ****	0.086	0.032	0.050	**0.345 ****	0.056
Li. sp. 'yellow chin pseudonigricans'	**0.383 ****	**0.256 ****	**0.210 ***	-0.019	**0.467 ****	-0.012
Pa. sauvagei	**0.447 ****	**0.341 ****	**0.259 ****	0.137 .	**0.475 ****	0.136 .
Pa. sp. 'short snout scraper'	**0.399 ****	**0.406 ****	**0.376 ****	0.096 .	**0.574 ****	0.096 .

(c)	*A. alluaudi*	*Pa. cyaneus*	*N. omnicaeruleus*	*N. sp. 'unicuspid scraper'*	*N. rufocaudalis*	*P.* sp. ' pink anal'
	non-radiating	*radiation*				
Pa. cyaneus	**0.290 ****					
N. omnicaeruleus	**0.294 ****	-0.024				
N. sp. 'unicuspid scraper'	**0.981 *****	**0.419 ****	**0.333 ****			
N. rufocaudalis	**0.592 *****	**0.179 ****	0.072	**0.467 *****		
P. sp. ' pink anal'	**0.894 *****	**0.378 *****	**0.31 ****	-0.084	**0.364 ****	
P. pundamilia	**0.915 *****	**0.917 *****	**0.868 *****	**0.871 *****	**0.904 *****	**0.901 *****
P. nyererei	**0.970 *****	**0.444 *****	**0.372 ****	-0.019	**0.496 *****	0.056
Li. sp. 'yellow chin pseudonigricans'	**0.742 *****	**0.438 *****	**0.343 ****	0.060	**0.494 *****	0.092
Pa. sauvagei	**0.989 *****	**0.596 *****	**0.537 *****	0.059	**0.602 *****	0.066
Pa. sp. 'short snout scraper'	**1.000 *****	**0.730 *****	**0.724 *****	**0.239 ***	**0.740 *****	0.118 .

Table S2.1 (b, c) (continued)

P. pundamilia	*P. nyererei*	*Li.* sp. 'yellow chin pseudonigricans'	*Pa. sauvagei*
0.098			
0.316 **	0.121 .		
-0.015	0.028	0.093	
0.271 **	**0.226 ****	0.004	**0.128 ***

P. pundamilia	*P. nyererei*	*Li.* sp. 'yellow chin pseudonigricans'	*Pa. sauvagei*
0.862 ***			
0.475 ***	**0.175 ***		
0.865 ***	-0.041	**0.158 ***	
0.938 ***	**0.350 ****	**0.152 ***	**0.168 ***

Table S2.2 (a)
Variation in parasite prevalence and intensity (pooling *Cichlidogyrus* species) among host species at Makobe island, including both radiation members and *A. alluaudi*. Variation in infection within the radiation is reported in the main text, **Table 2.4**. The Minimum Adequate Model (in bold) was established by stepwise removal of non-significant variables (in grey), and the contribution of each fixed effect was assessed through LRT. Model fits were also compared through AIC.

(a)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Cichlidogyrus* spp. prevalence**						***Cichlidogyrus* spp. intensity**					
	1					431.20	1					1906.61
	species	**17**	**114.21**	**<0.001**	*******	**350.99**	**species**	**14**	**784.22**	**<0.001**	*******	**1150.39**
	species	17	113.96	<0.001	***	351.68	species	14	781.42	<0.001	***	1151.82
	depth	1	1.31	0.252			depth	1	0.58	0.447		
	depth	1	1.57	0.211		431.63	depth	1	3.38	0.066	.	1905.23
	depth	1	0.67	0.414		407.87	depth	1	17.22	<0.001	***	1259.16
	diet	5	33.76	<0.001	***		diet	4	654.08	<0.001	***	
	diet	5	34.66	<0.001	***	406.54	diet	4	640.24	<0.001	***	1274.37
	***Lamproglena monodi* prevalence**						***Lamproglena monodi* intensity**					
	1					434.11	1					796.50
	species	**17**	**73.14**	**<0.001**	*******	**394.96**	species	16	47.31	<0.001	***	781.19
	species	17	69.04	<0.001	***	396.94	**species**	**16**	**39.06**	**0.001**	******	**773.77**
	depth	1	0.02	0.883			**depth**	**1**	**9.42**	**0.002**	******	
	depth	1	4.12	0.042	*	431.99	depth	1	17.68	<0.001	***	780.82
	depth	1	6.04	0.014	*	410.06	depth	1	13.65	<0.001	***	781.63
	diet	5	31.93	<0.001	***		diet	4	7.19	0.126		
	diet	5	30.02	<0.001	***	414.09	diet	4	11.22	0.024	*	793.29
	***Ergasilus lamellifer* prevalence**						***Ergasilus lamellifer* intensity**					
	1					**146.64**	**1**					**40.00**
	species	17	11.85	0.809		168.80	species	10	0.00	1.000		60.00
	species	17	11.14	0.849		169.91	species	10	0.00	1.000		62.00
	depth	1	0.88	0.347			depth	1	0.00	1.000		
	depth	1	1.60	0.207		147.05	depth	1	0.00	1.000		42.00
	depth	1	0.98	0.323		155.87	depth	1	0.00	1.000		48.00
	diet	5	1.17	0.947			diet	3	0.00	1.000		
	diet	5	1.79	0.877		154.85	diet	3	0.00	1.000		45.00
	Glochidia prevalence						**Glochidia intensity**					
	1					**290.72**	**1**					**170.34**
	species	17	24.85	0.098		299.87	species	11	7.73	0.737		184.61
	species	17	25.60	0.082	.	301.13	species	11	7.33	0.772		185.12
	depth	1	0.75	0.387			depth	1	1.49	0.223		
	depth	1	0.00	0.968		292.72	depth	1	1.89	0.169		170.45
	depth	1	0.29	0.591		298.94	depth	1	1.07	0.301		173.32
	diet	5	3.78	0.582			diet	3	3.13	0.372		
	diet	5	3.49	0.625		297.23	diet	3	3.95	0.267		172.39
	Nematodes prevalence						**Nematodes intensity**					
	1					244.46	1					2792.45
	species	**17**	**55.77**	**<0.001**	*******	**222.69**	species	15	1837.40	<0.0001	***	985.03
	species	17	50.02	<0.001	***	223.26	**species**	**15**	**1535.70**	**<0.001**	*******	**887.04**
	depth	1	1.43	0.232			**depth**	**1**	**100.00**	**<0.001**	*******	
	depth	1	7.18	0.007	**	239.28	depth	1	401.76	<0.001	***	2392.69
	depth	1	238.40	0.002	**	243.23	depth	1	427.28	<0.001	***	1653.55
	diet	5	235.28	0.302			diet	4	747.14	<0.001	***	
	diet	5	4.06	0.541		250.40	diet	4	721.62	<0.001	***	2078.83

Table S2.2 (b)
Variation in parasite prevalence and intensity (pooling *Cichlidogyrus* species) among host species at Makobe island, within the radiation, considering only host species represented by at least 10 individuals. Variation in infection within the radiation is reported in **Table 2.4**. The Minimum Adequate Model (in bold) was established by stepwise removal of non-significant variables (in grey), and the contribution of each fixed effect was assessed through LRT. Model fits were also compared through AIC.

(b)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Cichlidogyrus* spp. prevalence**						***Cichlidogyrus* spp. intensity**					
	1					379.40	1					985.70
	species	**10**	**86.16**	**<0.0001**	*******	**313.24**	**species**	**9**	**183.54**	**<0.0001**	*******	**820.15**
	species	10	86.94	<0.0001	***	313.39	species	9	140.31	<0.0001	***	821.96
	depth	1	1.85	0.174			depth	1	0.19	0.663		
	depth	1	1.06	0.303		380.34	depth	1	43.43	<0.0001	***	944.27
	depth	1	0.79	0.374		366.92	depth	1	19.85	<0.0001	***	919.81
	diet	2	17.42	<0.001	***		diet	2	28.46	<0.0001	***	
	diet	2	17.69	<0.001	***	365.71	diet	2	52.04	<0.0001	***	937.65
	***Lamproglena monodi* prevalence**						***Lamproglena monodi* intensity**					
	1					366.09	1					715.93
	species	**10**	**39.31**	**<0.0001**	*******	**346.78**	species	10	30.86	0.001	***	705.07
	species	10	26.25	<0.001	***	348.77	species	10	11.38	0.329		695.51
	depth	1	0.01	0.922			depth	1	11.56	0.001	***	
	depth	1	13.08	<0.001	***	355.02	**depth**	**1**	**31.04**	**<0.0001**	*******	**686.88**
	depth	1	10.49	0.001	**	355.24	depth	1	24.85	<0.0001	***	690.81
	diet	2	3.78	0.151			diet	2	0.08	0.961		
	diet	2	6.36	0.042	*	363.73	diet	2	6.27	0.043	*	713.66
	***Ergasilus lamellifer* prevalence**						***Ergasilus lamellifer* intensity**					
	1					**130.07**	**1**					**36.00**
	species	10	9.16	0.517		140.91	species	8	0.00	1.000		52.00
	species	10	8.88	0.543		142.20	species	8	0.00	1.000		54.00
	depth	1	0.71	0.400			depth	1	0.00	1.000		
	depth	1	0.99	0.320		131.08	depth	1	0.00	1.000		38.00
	depth	1	0.18	0.675		133.87	depth	1	0.00	1.000		42.00
	diet	2	1.21	0.545			diet	2	0.00	1.000		
	diet	2	2.03	0.363		132.05	diet	2	0.00	1.000		40.00
	Glochidia prevalence						**Glochidia intensity**					
	1					**248.52**	**1**					**149.49**
	species	10	18.07	0.054	.	250.46	species	8	7.30	0.505		158.19
	species	10	19.25	0.037	*	251.14	species	8	6.26	0.618		159.38
	depth	1	1.32	0.251			depth	1	0.81	0.367		
	depth	1	0.13	0.715		250.39	depth	1	1.85	0.174		149.64
	depth	1	0.03	0.871		252.49	depth	1	0.58	0.446		151.36
	diet	2	1.90	0.388			diet	2	2.29	0.319		
	diet	2	2.00	0.367		250.52	diet	2	3.56	0.169		149.93
	Nematodes prevalence						**Nematodes intensity**					
	1					176.67	1					1928.56
	species	9	**33.34**	**<0.001**	*******	**161.34**	**species**	**9**	**1370.80**	**0.000**	*******	**575.80**
	species	9	33.29	<0.001	***	163.30	**species**	**9**	**956.59**	**0.000**	*******	**574.82**
	depth	1	0.04	0.850			**depth**	**1**	**2.99**	**0.084**	**.**	
	depth	1	0.08	0.779		178.59	depth	1	417.15	<0.0001	***	1513.40
	depth	1	0.22	0.640		175.18	depth	1	207.80	<0.0001	***	1117.36
	diet	2	7.41	0.025	*		diet	2	400.04	<0.0001	***	
	diet	2	7.27	0.026	*	173.41	diet	2	609.39	<0.0001	***	1323.17

Table S2.2 (c)
Variation in parasite prevalence and intensity (pooling *Cichlidogyrus* species) among host species at Makobe island, including both radiation members and *A. alluaudi*, considering only host species represented by at least 10 individuals. The Minimum Adequate Model (in bold) was established by stepwise removal of non-significant variables (in grey), and the contribution of each fixed effect was assessed through LRT. Model fits were also compared through AIC.

(c)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Cichlidogyrus* spp. prevalence**						***Cichlidogyrus* spp. intensity**					
	1					396.58	1					1736.08
	species	**11**	**103.33**	**<0.0001**	*******	**315.24**	**species**	**10**	**768.89**	**<0.0001**	*******	**987.19**
	species	11	104.82	<0.0001	***	315.39	species	10	769.49	<0.0001	***	988.52
	depth	1	1.85	0.174			depth	1	0.67	0.413		
	depth	1	0.37	0.545		398.21	depth	1	0.07	0.788		1738.01
	depth	1	0.79	0.374		368.92	depth	1	5.99	0.014	*	1100.71
	diet	2	35.30	<0.0001	***		diet	3	643.31	<0.0001	***	
	diet	3	34.87	0.000	***	367.71	diet	3	637.39	<0.0001	***	1104.69
	***Lamproglena monodi* prevalence**						***Lamproglena monodi* intensity**					
	1					398.39	1					719.12
	species	**11**	**64.00**	**<0.0001**	*******	**356.39**	species	11	32.05	0.001	***	709.07
	species	11	56.58	<0.001	***	358.39	species	11	12.14	0.353		699.51
	depth	1	0.01	0.922			depth	1	11.56	0.001	***	
	depth	1	7.16	0.007	**	393.24	**depth**	**1**	**31.47**	**<0.0001**	*******	**689.65**
	depth	1	9.32	0.002	**	366.02	depth	1	24.85	<0.0001	***	694.81
	diet	3	33.22	0.000	***		diet	3	0.84	0.839		
	diet	3	31.06	0.000	***	373.34	diet	3	7.46	0.059	.	717.66
	***Ergasilus lamellifer* prevalence**						***Ergasilus lamellifer* intensity**					
	1					**137.68**	**1**					**38.00**
	species	11	9.17	0.607		150.51	species	9	0.00	1.000		56.00
	species	11	9.31	0.594		150.62	species	9	0.00	1.000		58.00
	depth	1	1.89	0.169			depth	1	0.00	1.000		
	depth	1	1.75	0.186		137.93	depth	1	0.00	1.000		40.00
	depth	1	0.70	0.404		142.95	depth	1	0.00	1.000		46.00
	diet	3	0.98	0.807			diet	3	0.00	1.000		
	diet	3	2.03	0.566		141.65	diet	3	0.00	1.000		44.00
	Glochidia prevalence						**Glochidia intensity**					
	1					**267.65**	**1**					**16.33**
	species	11	18.65	0.068	.	271.01	species	9	7.42	0.593		170.91
	species	11	19.30	0.056	.	272.22	species	9	6.39	0.701		171.42
	depth	1	0.79	0.375			depth	1	1.49	0.223		
	depth	1	0.13	0.714		269.52	depth	1	2.52	0.112		159.81
	depth	1	0.05	0.819		273.02	depth	1	1.12	0.289		163.53
	diet	3	2.50	0.475			diet	3	2.28	0.516		
	diet	3	2.58	0.461		271.07	diet	3	3.68	0.298		162.65
	Nematodes prevalence						**Nematodes intensity**					
	1					190.51	1					2010.06
	species	10	**33.71**	**<0.001**	*******	**176.79**	**species**	**10**	**1404.70**	**<0.0001**	*******	**625.40**
	species	10	33.58	<0.001	***	178.73	species	10	988.32	<0.0001	***	623.98
	depth	1	0.07	0.798			depth	1	3.43	0.064	.	
	depth	1	0.19	0.661		192.31	depth	1	419.76	<0.0001	***	1592.29
	depth	1	0.50	0.482		190.37	depth	1	224.39	<0.0001	***	1150.38
	diet	3	1.94	0.047	*		diet	3	447.92	<0.0001	***	
	diet	3	7.64	0.054	.	188.86	diet	3	643.29	<0.0001	***	1372.77

Table S2.3 (a)
Variation in parasites prevalence and intensity (pooling *Cichlidogyrus* species) among host species at Makobe island, within the radiation. Fixed effects included fish body length (SL), in addition to host species, diet, and water depth (as in **Table 2.4** and **Table S2.2**). The Minimum Adequate Model (in bold) was established by stepwise removal of non-significant variables (in grey), and the contribution of each fixed effect was assessed through LRT. Model fits were also compared through AIC.

(a)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Cichlidogyrus* spp. prevalence**						***Cichlidogyrus* spp. intensity**					
	1					414.57	1					1170.92
	species	16	16.00	<0.0001	***	348.99	species	13	213.57	<0.0001	***	983.35
	depth	1	2.75	0.097		413.82	depth	1	82.56	<0.0001	***	1090.37
	SL	1	5.03	0.025	*	411.54	SL	1	59.48	<0.0001	***	1113.44
	diet	5	25.76	<0.0001	***	398.82	diet	3	69.59	<0.0001	***	1107.33
	species	16	96.15	<0.0001	***	349.68	species	13	139.14	<0.0001	***	977.23
	depth	1	1.31	0.252			depth	1	8.13	0.004	**	
	species	**16**	**97.10**	**<0.0001**	*******	**346.44**	species	13	178.99	<0.0001	***	960.46
	SL	**1**	**4.55**	**0.033**	*****		SL	1	24.90			
	depth	1	2.56	0.110		410.98	depth	1	63.06	<0.0001	***	1052.38
	SL	1	4.84	0.028	*		SL	1	39.99	<0.0001	***	
	depth	1	0.37	0.545		400.45	depth	1	43.09	<0.0001	***	1066.25
	diet	5	23.37	<0.001	***		diet	3	30.12	<0.0001	***	
	diet	5	23.80	<0.001	***	397.74	diet	3	42.51	<0.0001	***	1076.93
	SL	1	3.07	0.080	.		SL	1	32.41	<0.0001	***	
	species	16	94.71	<0.0001	***	348.27	**species**	**13**	**130.60**	**<0.0001**	*******	**947.78**
	depth	1	0.16	0.686			**depth**	**1**	**14.68**	**<0.001**	*******	
	SL	1	3.41	0.065	.		**SL**	**1**	**31.45**	**<0.0001**	*******	
	***Lamproglena monodi* prevalence**						***Lamproglena monodi* intensity**					
	1					401.42	1					793.29
	species	16	48.06	<0.0001	***	385.36	species	15	46.10	<0.0001	***	777.19
	depth	1	8.05	0.005	**	395.37	depth	1	17.40	<0.0001	***	777.88
	SL	1	1.55	0.214		401.87	SL	1	12.55	<0.001	***	782.73
	diet	5	9.88	0.079	.	401.53	diet	4	10.00	0.040	*	791.29
	species	16	40.13	0.001	***	387.24	species	15	38.12	0.001	***	769.77
	depth	1	0.12	0.735			depth	1	9.42	0.002	**	
	species	**16**	**50.77**	**0.000**	*******	**383.10**	**species**	**15**	**59.78**	**<0.0001**	*******	**752.95**
	SL	**1**	**4.26**	**0.039**	*****		**SL**	**1**	**26.24**	**<0.0001**	*******	
	depth	1	8.55	0.003	**	395.32	depth	1	19.25	<0.0001	***	765.49
	SL	1	2.05	0.152			SL	1	14.40	<0.001	***	
	depth	1	5.88	0.015	*	397.65	depth	1	13.75	<0.001	***	779.54
	diet	5	7.72	0.172			diet	4	6.34	0.175		
	diet	5	16.42	0.006	**	395.45	diet	4	24.71	<0.0001	***	766.03
	SL	1	8.09	0.004	**		SL	1	27.26	<0.0001	***	
	species	16	42.31	0.000	***	385.01	species	15	40.91	<0.001	***	754.57
	depth	1	0.09	0.769			depth	1	0.38	0.536		
	SL	1	4.23	0.040	*		SL	1	17.19	<0.0001	***	

Table S2.3 (a) (continued)

(a)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Ergasilus lamellifer* prevalence**						***Ergasilus lamellifer* intensity**					
	1					**139.04**	**1**					**38.00**
	species	16	11.85	0.754		159.19	species	9	0.00	1.000		56.00
	depth	1	0.89	0.346		140.15	depth	1	0.00	1.000		40.00
	SL	1	0.02	0.879		141.01	SL	1	0.00	1.000		40.00
	diet	5	1.91	0.861		147.13	diet	2	0.00	1.000		42.00
	species	16	11.11	0.803		161.04	species	9	0.00	1.000		58.00
	depth	1	0.15	0.699			depth	1	0.00	1.000		
	species	16	15.08	0.519		157.93	species	9	0.00	1.000		58.00
	SL	1	3.26	0.071	.		SL	1	0.00	1.000		
	depth	1	0.89	0.345		142.12	depth	1	0.00	1.000		42.00
	SL	1	0.03	0.871			SL	1	0.00	1.000		
	depth	1	0.40	0.526		148.72	depth	1	0.00	1.000		44.00
	diet	5	1.43	0.922			diet	2	0.00	1.000		
	diet	5	2.43	0.787		148.58	diet	2	0.00	1.000		44.00
	SL	1	0.54	0.461			SL	1	0.00	1.000		
	species	16	14.31	0.576		159.82	species	9	0.00	1.000		60.00
	depth	1	0.12	0.734			depth	1	0.00	1.000		
	SL	1	3.22	0.073	.		SL	1	0.00	1.000		
	Glochidia prevalence						**Glochidia intensity**					
	1					**271.56**	**1**					159.52
	species	16	24.24	0.084	.	279.32	species	10	7.63	0.665		171.89
	depth	1	0.00	0.988		273.56	depth	1	1.29	0.256		160.23
	SL	1	0.09	0.765		273.47	SL	1	3.73	0.054	.	157.80
	diet	5	3.40	0.639		278.16	diet	1	3.85	0.146		159.67
	species	16	25.46	0.062	.	280.09	species	10	7.15	0.711		173.08
	depth	1	1.23	0.268			depth	1	0.81	0.367		
	species	16	25.27	0.065	.	280.20	species	10	9.14	0.518		168.65
	SL	1	1.12	0.289			SL	1	5.24	0.022	*	
	depth	1	0.00	0.997		275.47	depth	1	2.42	0.120		157.38
	SL	1	0.09	0.765			SL	1	4.86	0.028	*	
	depth	1	0.19	0.667		279.98	depth	1	0.56	0.454		161.11
	diet	5	3.58	0.611			diet	2	3.12	0.210		
	diet	5	3.57	0.613		279.90	diet	2	5.00	0.082	.	156.79
	SL	1	0.26	0.609			SL	1	4.88	0.027	*	
	species	16	25.89	0.056	.	281.58	species	10	7.42	0.685		169.96
	depth	1	0.62	0.432			depth	1	0.69	0.405		
	SL	1	0.51	0.475			SL	1	5.12	0.024	*	

Table S2.3 (a) (continued)

(a)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	Nematodes prevalence						**Nematodes intensity**					
	1					230.68	**1**					2698.37
	species	16	55.46	<0.0001	***	207.23	species	14	1790.90	<0.0001	***	935.43
	depth	1	7.06	0.008	**	225.62	depth	1	379.07	<0.0001	***	2321.29
	SL	1	5.69	0.017	*	226.99	SL	1	159.12	<0.0001	***	2541.24
	diet	5	5.45	0.364		235.24	diet	3	675.14	<0.0001	***	2029.23
	species	16	49.35	<0.0001	***	208.27	**species**	**14**	**1495.37**	**<0.0001**	*******	853.92
	depth	1	0.96	0.328			**depth**	**1**	**83.51**	**<0.0001**	*******	
	species	**16**	**57.60**	**<0.0001**	*******	**201.40**	species	1	1632.30	<0.0001	***	936.93
	SL	**1**	**7.83**	**0.005**	******		SL	1	0.50	0.479		
	depth	1	5.06	0.024	*	223.93	depth	1	237.76	<0.0001	***	2305.49
	SL	1	3.69	0.055	.		SL	1	17.81	<0.0001	***	
	depth	1	7.78	0.005	**	229.46	depth	1	410.58	<0.0001	***	1620.64
	diet	5	6.16	0.291			diet	3	706.65	<0.0001	***	
	diet	5	12.33	0.031	*	224.66	diet	3	738.29	<0.0001	***	1808.96
	SL	1	12.58	<0.001	***		SL	1	222.27	<0.0001	***	
	species	16	54.07	<0.0001	***	201.86	species	14	1477.69	<0.0001	***	855.80
	depth	1	1.54	0.215			depth	1	83.13	<0.0001	***	
	SL	1	8.42	0.004	**		SL	1	0.12	0.724		

Table S2.3 (b)
Variation in parasites prevalence and intensity (pooling *Cichlidogyrus* species) among host species at Makobe island, including both radiation members and *Astatoreochromis alluaudi*. Fixed effects included fish body length (SL), in addition to host species, diet, and water depth (as in **Table 2.4** and **Table S2.2**). The Minimum Adequate Model (in bold) was established by stepwise removal of non-significant variables (in grey), and the contribution of each fixed effect was assessed through LRT. Model fits were also compared through AIC.

(b)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Cichlidogyrus* spp. prevalence**						***Cichlidogyrus* spp. intensity**					
	1					431.20	1					1906.61
	species	17	114.21	<0.0001	***	350.98	species	14	784.22	<0.0001	***	1150.39
	depth	1	1.57	0.211		431.63	depth	1	3.38	0.066	.	1905.23
	SL	1	8.69	0.003	**	424.51	SL	1	281.07	<0.0001	***	1627.54
	diet	5	34.66	<0.0001	***	406.54	diet	4	640.25	<0.0001	***	1274.37
	species	17	113.96	<0.0001	***	351.67	species	14	781.42	<0.0001	***	1151.82
	depth	1	1.31	0.252			depth	1	0.58	0.447		
	species	**17**	**110.08**	**<0.0001**	*******	**348.43**	**species**	**14**	**553.98**	**<0.0001**	*******	**1101.60**
	SL	**1**	**4.55**	**0.033**	*****		**SL**	**1**	**50.83**	**<0.0001**	*******	
	depth	1	1.63	0.201		**424.87**	depth	1	0.41	0.519		1629.13
	SL	1	8.75	0.003	**		SL	1	278.11	<0.0001	***	
	depth	1	0.67	0.414		407.87	depth	1	17.22	<0.0001	***	1259.16
	diet	5	33.76	<0.0001	***		diet	4	654.08	<0.0001	***	
	diet	5	28.44	<0.0001	***	406.07	diet	4	415.65	<0.0001	***	1219.90
	SL	1	2.47	0.116			SL	1	56.48	<0.0001	***	
	species	17	108.61	<0.0001	***	350.27	species	14	556.58	<0.0001	***	1100.54
	depth	1	0.16	0.686			depth	1	3.02	0.082	.	
	SL	1	3.41	0.065	.		SL	1	53.28	<0.0001	***	
	***Lamproglena monodi* prevalence**						***Lamproglena monodi* intensity**					
	1					434.11	1					796.50
	species	17	73.14	<0.0001	***	394.96	species	16	47.31	<0.0001	***	781.19
	depth	1	4.12	0.042	*	431.99	depth	1	17.68	<0.0001	***	780.82
	SL	1	0.00	0.999		436.11	SL	1	12.16	<0.001	***	786.34
	diet	5	30.02	<0.0001	***	414.09	diet	4	11.22	0.024	*	793.29
	species	17	69.04	<0.0001	***	396.94	species	16	39.06	0.001	**	773.77
	depth	1	0.02	0.883			depth	1	9.42	0.002	**	
	species	**17**	**77.18**	**<0.0001**	*******	**392.93**	**species**	**16**	**61.39**	**<0.0001**	*******	**756.95**
	SL	**1**	**4.03**	**0.045**	*****		**SL**	**1**	**26.24**	**<0.0001**	*******	
	depth	1	4.12	0.042	*	433.99	depth	1	19.56	<0.0001	***	768.78
	SL	1	0.00	0.976			SL	1	14.04	<0.001	***	
	depth	1	6.04	0.014	*	410.06	depth	1	13.65	<0.001	***	781.63
	diet	5	31.93	<0.0001	***		diet	4	7.19	0.126		
	diet	5	38.63	<0.0001	***	407.48	diet	4	26.24	<0.0001	***	768.10
	SL	1	8.62	0.003	**		SL	1	27.19	<0.0001	***	
	species	17	73.27	<0.0001	***	394.72	species	16	42.21	<0.001	***	758.57
	depth	1	0.21	0.645			depth	1	0.38	0.536		
	SL	1	4.22	0.040	*		SL	1	17.19	<0.0001	***	

Table S2.3 (b) (continued)

(b)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	***Ergasilus lamellifer* prevalence**						***Ergasilus lamellifer* intensity**					
	1					**146.64**	**1**					**40.00**
	species	17	11.85	0.809		168.80	species	10	0.00	1.000		60.00
	depth	1	1.60	0.207		147.05	depth	1	0.00	1.000		42.00
	SL	1	0.12	0.733		148.53	SL	1	0.00	1.000		42.00
	diet	5	1.79	0.877		154.85	diet	3	0.00	1.000		46.00
	species	17	11.14	0.849		169.91	species	10	0.00	1.000		62.00
	depth	1	0.88	0.347			depth	1	0.00	1.000		
	species	17	15.88	0.532		166.64	species	1	0.00	1.000		62.00
	SL	1	4.15	0.042	*		SL	1	0.00	1.000		
	depth	1	1.57	0.210		148.96	depth	1	0.00	1.000		44.00
	SL	1	0.09	0.764			SL	1	0.00	1.000		
	depth	1	0.98	0.323		155.87	depth	1	0.00	1.000		48.00
	diet	5	1.17	0.947			diet	3	0.00	1.000		
	diet	5	2.54	0.771		155.99	diet	3	0.00	1.000		48.00
	SL	1	0.86	0.353			SL	1	0.00	1.000		
	species	17	14.37	0.641		168.59	species	10	0.00	1.000		64.00
	depth	1	0.05	0.816			depth	1	0.00	1.000		
	SL	1	3.32	0.068	.		SL	1	0.00	1.000		
	Glochidia prevalence						**Glochidia intensity**					
	1					**290.72**	**1**					170.34
	species	17	24.85	0.098		299.87	species	11	7.73	0.737		184.61
	depth	1	0.00	0.968		292.72	depth	1	1.89	0.169		170.45
	SL	1	0.00	0.987		292.72	**SL**	**1**	**3.93**	**0.048**	*****	**168.42**
	diet	5	3.49	0.625		297.23	diet	3	3.95	0.267		172.39
	species	17	25.60	0.082	.	301.13	species	11	7.33	0.772		185.12
	depth	1	0.75	0.387			depth	1	1.49	0.223		
	species	17	26.07	0.073	.	300.65	species	11	9.13	0.610		181.28
	SL	1	1.23	0.268			SL	1	5.33	0.021	*	
	depth	1	0.00	0.968		294.72	depth	1	3.14	0.076	.	167.27
	SL	1	0.00	0.987			SL	1	5.18	0.023	*	
	depth	1	0.29	0.591		298.94	depth	1	1.07	0.301		173.32
	diet	5	3.78	0.582			diet	3	3.13	0.372		
	diet	5	3.77	0.583		298.95	diet	3	5.11	0.164		169.30
	SL	1	0.28	0.597			SL	1	5.09	0.024	*	
	species	17	26.37	0.068	.	302.35	species	11	8.05	0.709		181.22
	depth	1	0.30	0.584			depth	1	2.06	0.151		
	SL	1	0.78	0.378			SL	1	5.90	0.015	*	

Table S2.3 (b) (continued)

(b)	factors	df	LRT	p		AIC	factors	df	LRT	p		AIC
	Nematodes prevalence						Nematodes intensity					
	1					244.46	**1**					2792.45
	species	17	55.77	<0.0001	***	222.69	species	15	1837.40	<0.0001	***	985.03
	depth	1	7.18	0.007	**	239.28	depth	1	401.76	<0.0001	***	2392.69
	SL	1	7.54	0.006	**	238.92	SL	1	124.00	<0.0001	***	2670.46
	diet	5	4.06	0.541		250.40	diet	4	721.62	<0.0001	***	2078.83
	species	17	50.02	<0.0001	***	223.26	**species**	**15**	**1535.70**	**<0.0001**	*******	**887.04**
	depth	1	1.43	0.232			**depth**	**1**	**100.00**	**<0.0001**	*******	
	species	**17**	**58.26**	**<0.0001**	*******	**214.66**	species	15	1714.48	<0.0001	***	985.98
	SL	**1**	**10.03**	**0.002**	******		SL	1	1.05	0.306		
	depth	1	5.45	0.020	*	235.47	depth	1	291.70	<0.0001	***	2380.76
	SL	1	5.81	0.016	*		SL	1	13.93	<0.001	***	
	depth	1	9.16	0.002	**	243.23	depth	1	427.28	<0.0001	***	1653.55
	diet	5	6.05	0.302			diet	4	747.14	<0.0001	***	
	diet	5	10.02	0.075	.	238.90	diet	4	808.74	<0.0001	***	1869.72
	SL	1	13.49	<0.001	***		SL	1	211.11	<0.0001	***	
	species	17	55.68	<0.0001	***	213.80	species	15	1521.78	<0.0001	***	888.98
	depth	1	2.86	0.091			depth	1	99.00	<0.0001	***	
	SL	1	11.46	0.001	***		SL	1	0.06	0.806		

Table S2.4 (a)
Differences in ectoparasite community (pooling *Cichlidogyrus* species) between cichlid host species at Makobe Island in 2010 and 2014, expressed as R values based on zero-adjusted Bray-Curtis distances. Ectoparasite community composition of *Astatoreochromis alluaudi* (non-radiating lineage) differed from all radiation members. Within the radiation, there were 71 significant differences between host species (out of 105). ANOSIM pairwise comparisons (Benjamini-Hochberg correction), 9999 permutations.

(a)	*A. alluaudi*	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*	*M. mbipi*
	non-radiating	radiation				
La. sp. 'stone'	**0.927 *****					
Pa. chilotes	**0.781 *****	-0.044				
Pa. cyaneus	**0.335 *****	**0.552 *****	**0.405 *****			
M. lutea	**0.585 *****	**0.397 *****	**0.295 *****	0.037		
M. mbipi	**0.373 *****	**0.352 *****	**0.292 *****	0.038	**0.131 ***	
N. gigas	**0.461 *****	**0.596 *****	**0.447 *****	0.006	**0.097 ***	0.007
N. omnicaeruleus	**0.380 *****	**0.211 ****	**0.175 ****	0.021	0.080 .	-0.007
N. sp. 'unicuspid scraper'	**0.796 *****	0.093 .	**0.138 ****	**0.225 *****	0.083	**0.238 *****
N. rufocaudalis	**0.594 *****	**0.437 *****	**0.290 *****	**0.210 *****	**0.295 *****	0.012
P. sp. ' pink anal'	**0.819 *****	-0.003	0.045	**0.379 *****	**0.300 *****	**0.235 *****
P. pundamilia	**0.792 *****	-0.068	0.063	**0.378 *****	**0.295 *****	**0.274 *****
P. nyererei	**0.820 *****	0.003	**0.125 ***	**0.302 *****	**0.148 ***	**0.311 *****
Li. melanopterus	**0.899 *****	0.000	-0.077	**0.454 *****	**0.321 ****	**0.273 ****
Pa. sauvagei	**0.954 *****	0.072	**0.111 ***	**0.574 *****	**0.369 *****	**0.527 *****
Pa. sp. 'short snout scraper'	**0.948 *****	0.094 .	**0.142 ****	**0.596 *****	**0.368 *****	**0.592 *****

Table S2.4 (a) (continued)

	N. gigas	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. ' pink anal'	*P. pundamilia*	*P. nyererei*	*Li. melanopterus*	*Pa. sauvagei*
radiation (cont,)									
	-0.002								
	0.205 **	**0.142 *****							
	0.201 **	0.000	**0.264 *****						
	0.367 ***	**0.127 ****	**0.064 ***	**0.244 *****					
	0.329 ***	**0.202 *****	**0.08 ****	**0.224 *****	-0.044				
	0.253 ***	**0.226 *****	-0.015	**0.316 *****	0.033	**0.066 ****			
	0.598 ***	0.131 .	0.085	**0.288 ****	0.023	-0.011	0.053		
	0.606 ***	**0.358 *****	**0.093 ***	**0.614 *****	**0.099 ***	0.009	0.000	**0.163 ***	
	0.625 ***	**0.467 *****	**0.229 *****	**0.636 *****	**0.202 ****	**0.141 ***	**0.16 ****	0.117 .	0.019

Table S2.4 (b)
Differences in ectoparasite community (pooling *Cichlidogyrus* species) between cichlid host species at Makobe Island in 2010 and 2014, expressed as R values based on Jaccard indices. Ectoparasite community composition of *Astatoreochromis alluaudi* (non-radiating lineage) differed from most radiation members. Within the radiation, there were 48 significant differences between host species (out of 105). ANOSIM pairwise comparisons (Benjamini-Hochberg correction), 9999 permutations.

(b)	*A. alluaudi*	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*	*M. mbipi*
	non-radiating	radiation				
La. sp. 'stone'	**0.283 *** **					
Pa. chilotes	**0.194 ** **	-0.016				
Pa. cyaneus	**0.339 *** **	**0.240 ** **	**0.231 *** **			
M. lutea	**0.335 *** **	**0.135 * **	**0.139 ** **	-0.003		
M. mbipi	0.032	0.083	**0.118 * **	0.034	0.006	
N. gigas	**0.353 *** **	**0.174 * **	**0.168 *** **	-0.005	-0.015	-0.004
N. omnicaeruleus	**0.081 * **	**0.199 * **	**0.157 * **	-0.006	0.000	0.013
N. sp. 'unicuspid scraper'	**0.309 *** **	0.168 .	**0.203 ** **	-0.060	-0.077	**0.099 ** **
N. rufocaudalis	-0.004	**0.330 ** **	**0.180 ** **	**0.329 *** **	**0.318 *** **	0.052 .
P. sp. ' pink anal'	**0.220 *** **	0.003	0.022	0.008	-0.069	**0.072 * **
P. pundamilia	0.016	-0.033	0.000	-0.171	-0.199	-0.053
P. nyererei	**0.221 *** **	0.126	**0.182 * **	-0.120	-0.139	0.075
Li. melanopterus	**0.226 * **	-0.019	-0.032	**0.314 * **	**0.219 * **	0.094
Pa. sauvagei	**0.620 *** **	0.100 .	**0.156 ** **	**0.341 *** **	**0.185 *** **	**0.381 *** **
Pa. sp. 'short snout scraper'	**0.711 *** **	**0.102 * **	**0.177 ** **	**0.447 *** **	**0.278 *** **	**0.494 *** **

Table S2.4 (b) (continued)

N. gigas	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. ' pink anal'	*P. pundamilia*	*P. nyererei*	*Li. melanopterus*	*Pa. sauvagei*
radiation (cont.)								
0.008								
-0.066	**0.066 ***							
0.343 ***	0.044	**0.274 *****						
-0.030	**0.088 ***	0.064 .	**0.180 *****					
-0.197	-0.040	-0.059	0.013	-0.038				
-0.141	0.055	-0.047	**0.218 *****	0.080	**0.048 ****			
0.291 *	0.096	0.180	0.187 .	-0.015	-0.037	0.153		
0.280 ***	**0.373 *****	**0.199 ****	**0.546 *****	**0.075 ***	0.023	0.114 .	0.179 .	
0.379 ***	**0.491 *****	**0.315 *****	**0.627 *****	**0.132 ***	0.100	**0.227 ****	**0.183 ***	-0.001

Table S2.5
Temporal consistency in ectoparasite prevalence and intensity (pooling *Cichlidogyrus* species) among host species at Makobe island, **(a)** within the radiation, **(b)** including both radiation members and *Astatoreochromis alluaudi*. The Minimum Adequate Model (MAM) was established by stepwise removal of non-significant variables (not shown), and the contribution of each fixed effect was assessed through LRT. Species differences in infection were consistent across time for all parasite taxa, except *Cichlidogyrus* spp. prevalence, *Lamproglena monodi* intensity.

(a)

factors	df	LRT	p		factors	df	LRT	p	
***Cichlidogyrus* spp. prevalence**					***Cichlidogyrus* spp. intensity**				
species	14	35.94	0.001	**	species	13	172.65	<0.0001	***
depth	1	4.49	0.034	*	depth	1	4.27	0.039	*
year:species	15	35.97	0.002	**	year	1	28.10	<0.0001	***
***Lamproglena monodi* prevalence**					***Lamproglena monodi* intensity**				
species	14	66.76	<0.0001	***	species	14	107.78	<0.0001	
					depth	1	8.86	0.003	**
					year	1	7.00	0.008	**
					year:species	14	24.82	0.036	*
***Ergasilus lamellifer* prevalence**					***Ergasilus lamellifer* intensity**				
species	14	27.25	0.018	*	1				
year	1	7.86	0.005	**					
Glochidia prevalence					**Glochidia intensity**				
species	14	35.30	0.001	**	species	11	43.20	<0.0001	***
					year	1	31.84	<0.0001	***

(b)

factors	df	LRT	p		factors	df	LRT	p	
***Cichlidogyrus* spp. prevalence**					***Cichlidogyrus* spp. intensity**				
species	15	173.25	<0.0001	***	species	14	1035.31	<0.0001	***
depth	1	4.49	0.034	*	year	1	45.82	<0.0001	***
year:species	15	35.97	0.003	**					
***Lamproglena monodi* prevalence**					***Lamproglena monodi* intensity**				
species	15	89.10	<0.0001	***	species	15	109.31	<0.0001	***
					depth	1	8.56	0.003	**
					year	1	7.32	0.007	**
					year:species	15	25.12	0.048	*
***Ergasilus lamellifer* prevalence**					***Ergasilus lamellifer* intensity**				
species	15	27.17	0.027	*	1				
year	1	7.89	0.005	**					
Glochidia prevalence					**Glochidia intensity**				
species	15	37.76	0.001	***	species	12	43.09	<0.0001	***
					year	1	34.02	<0.0001	***

Table S2.6
Differences in *Cichlidogyrus* community between host species of the radiation at Makobe Island, expressed as R values based on **(a)** zero-adjusted Bray-Curtis distances, **(b)** Jaccard indices, **(c)** zero-adjusted Bray-Curtis distances of host species represented by at least 5 individuals, **(d)** Jaccard distances of host species represented by at least 5 individuals. *Cichlidogyrus* community composition Most radiation members at Makobe have similar *Cichlidogyrus* communities. ANOSIM pairwise comparisons (Benjamini-Hochberg correction), 9999 permutations.

(a)	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*	*M. mbipi*	*N. gigas*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. ' pink anal'	*P. pundamilia*
Pa. chilotes	0.125										
Pa. cyaneus	-0.073	0.344									
M. lutea	1.000	1.000	-0.100								
M. mbipi	0.176	0.025	0.355	0.516							
N. gigas	1.000	1.000	-0.036	0.000	0.620 .						
N. omnicaeruleus	-0.235	-0.235	-0.010	0.143	0.013	0.257					
N. sp. 'unicuspid scraper'	-0.133	-0.199	0.166	0.535 .	0.082	0.553 .	-0.016				
N. rufocaudalis	0.125	0.427	-0.153	-0.071	0.250	0.222	-0.077	0.111			
P. sp. ' pink anal'	-0.195	-0.152	0.132	0.234	0.066	0.375	-0.030	0.126	0.026		
P. pundamilia	-0.112	-0.228	0.154	0.346	0.006	**0.493 ***	-0.025	0.001	0.046	-0.025	
P. nyererei	0.256	0.056	0.440 .	0.548	0.022	**0.781 ***	0.129	0.149	0.272	0.098	0.038

(b)	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*	*M. mbipi*	*N. gigas*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. ' pink anal'	*P. pundamilia*
Pa. chilotes	-0.179										
Pa. cyaneus	-0.164	-0.088									
M. lutea	0.000	0.125	0.236								
M. mbipi	0.153	0.095	0.139	0.173							
N. gigas	-0.167	-0.009	0.036	0.167	0.262						
N. omnicaeruleus	-0.159	-0.130	-0.104	-0.039	0.002	-0.048					
N. sp. 'unicuspid scraper'	-0.186	-0.155	-0.138	0.029	0.038	-0.027	-0.037				
N. rufocaudalis	-0.071	-0.083	0.000	-0.036	0.004	0.111	-0.130	-0.143			
P. sp. ' pink anal'	-0.214	-0.142	-0.060	-0.247	0.075	-0.115	-0.004	0.056	-0.175		
P. pundamilia	-0.133	-0.142	-0.113	-0.201	-0.042	-0.076	-0.047	-0.009	-0.177	-0.063	
P. nyererei	0.338	0.250	0.281	0.166	-0.002	0.357	0.123	0.182	0.149	0.153	0.074

Table S2.6 (continued)

(c)	*Pa. chilotes*	*M. mbipi*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*P.* sp. 'pink anal'	*P. pundamilia*
M. mbipi	0.355					
N. omnicaeruleus	-0.010	0.013				
N. sp. 'unicuspid scraper'	0.166	0.082	-0.020			
P. sp. 'pink anal'	0.132	0.066	-0.030	0.126		
P. pundamilia	0.154	0.006	-0.030	0.001	-0.030	
P. nyererei	0.440	0.022	0.129	0.149	0.098	0.038

(d)	*Pa. chilotes*	*M. mbipi*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*P.* sp. 'pink anal'	*P. pundamilia*
M. mbipi	0.139					
N. omnicaeruleus	-0.100	0.002				
N. sp. 'unicuspid scraper'	-0.140	0.038	-0.040			
P. sp. 'pink anal'	-0.060	0.075	-0.000	0.056		
P. pundamilia	-0.110	-0.040	-0.050	-0.009	-0.060	
P. nyererei	0.281	-0.000	0.123	0.182	0.153	0.074

Contribution of each parasite taxon to lineage differences in parasite community

To investigate the contribution of each parasite taxon to the differences in infection profile among host species, we performed similarity percentages analysis (SIMPER, PAST 3.18, Hammer et al., 2001). At Makobe, the main contributor to the difference between radiation members and *A. alluaudi* was *Cichlidogyrus* spp. (pooling *Cichlidogyrus* species; 76.64%), being highly abundant in *A. alluaudi* (25.40±4.90 vs. 3.43±0.37). Nematodes were the second main contributor (17.51%): nearly absent in *A. alluaudi* but common in some radiation members (2.67±1.67 vs. 7.40±1.66).

Considering the species community of *Cichlidogyrus*, the main contributor to the difference between radiation members and *A. alluaudi* was *Cichlidogyrus longipenis* (56.55%), dominant in *A. alluaudi* from both sampling sites but nearly absent in radiation members (4.0±1.06 vs. 0.009±0.009). The difference between radiation members and *Ps. multicolor* was led by *Cichlidogyrus nyanza* (32.06%), dominant in radiation members but nearly absent in *Ps. multicolor* (1.24±0.13 vs 0.17±0.17). This morphospecies was also the second contributor to the difference between *A. alluaudi* and radiation members (20.27%; 0.17±0.11 vs. 1.24±0.13). At Sweya, the main contributors to the difference between the two non-radiating lineages were *Cichlidogyrus longipenis*, abundant in *A. alluaudi* and absent in *Ps. multicolor* (54.49%; 4.50±3.17 vs. 0.00±0.00) and *Cichlidogyrus bifurcatus*, absent in *A. alluaudi* and rarely present in *Ps. multicolor* (20.39%; 0.00±000 vs 1.00±0.44).

Table S2.7
Differences in *Cichlidogyrus* community between cichlid host species of the radiating and non-radiating lineages at Makobe, Sweya and Kissenda locations, expressed as R values based on **(a)** zero-adjusted Bray-Curtis distances (also reported in the main text, **Table 2.5**), **(b)** Jaccard indices, **(c)** zero-adjusted Bray-Curtis distances of host species represented by at least 5 individuals, **(d)** Jaccard distances of host species represented by at least 5 individuals. *Cichlidogyrus* community composition of *Astatoreochromis alluaudi* (non-radiating lineage) is similar at Makobe and Sweya. Most radiation members at Makobe have similar *Cichlidogyrus* communities, also similar to radiation members at Kissenda. ANOSIM pairwise comparisons (Benjamini-Hochberg correction), 9999 permutations.

(a)

			Ps. multicolor	*A. alluaudi*	*A. alluaudi*	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*
			Sweya		Makobe				
			non-radiating lineages			radiation lineage			
non-radiating	Sweya	*A. alluaudi*	**0.893 ***						
		A. alluaudi	**0.480 ****	0.393 .					
radiation lineage	Makobe	*La.* sp. 'stone'	0.344	0.834	0.357 .				
		Pa. chilotes	0.367	0.845 .	**0.625 ***	0.125			
		Pa. cyaneus	**0.688 ***	**0.924 ***	**0.775 ***	-0.073	0.344		
		M. lutea	0.604 .	0.972	0.750 .	1.000	1.000	-0.100	
		M. mbipi	**0.427 ***	**0.872 ****	**0.711 ****	0.176	0.025	0.355 .	0.516 .
		N. gigas	0.787 .	0.964 .	**0.759 ***	1.000	1.000 .	-0.036	0.000
		N. omnicaeruleus	**0.362 ***	**0.763 ***	**0.528 ****	-0.235	-0.235	-0.010	0.143
		N. sp. 'unicuspid scraper'	**0.543 ****	**0.875 ****	**0.795 ****	-0.133	-0.199	0.166	**0.535 ***
		N. rufocaudalis	0.601 .	0.919 .	**0.719 ***	0.125	0.427	-0.153	-0.071
		P. sp. 'pink anal'	0.171	0.700	**0.315 ****	-0.195	-0.152	0.132	0.234
		P. pundamilia	**0.560 ***	**0.853 ****	**0.770 ****	-0.297	-0.046	0.073	0.578 .
		P. nyererei	0.292 .	**0.795 ***	**0.523 ****	-0.112	-0.228	**0.154 ***	0.346 .
	Kissenda	*P.* sp. 'pundamilia-like'	0.120	0.811	**0.333 ***	0.125	0.398	0.615 .	0.333
		P. sp. 'nyererei-like'	**0.281 ***	**0.792 ***	**0.546 ****	0.256	0.056	0.440	0.548
		Pt. xenognathus	0.620 .	0.876 .	**0.667 ***	-0.125	0.352	-0.103	-0.417

Table S2.7 (a) (continued)

M. mbipi	*N. gigas*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. 'pink anal'	*P. pundamilia*	*P. nyererei*	*P.* sp. 'pundamilia-like'	*P.* sp. 'nyererei-like'
Makobe (cont.)								Kissenda	
radiation lineage (cont.)									
0.620 *									
0.013	0.257								
0.082	**0.553 ***	-0.016							
0.250 .	0.222	-0.077	0.111						
0.066	0.375	-0.030	0.126	0.026					
0.276	**0.639 ****	-0.102	-0.003	0.119	0.009				
0.006	**0.493 ****	-0.025	0.001 .	0.046	-0.025	-0.015			
0.211	0.796	0.097	0.330	0.491 .	-0.083	0.491	0.045		
0.022 .	0.781 .	0.129	0.149	0.272	0.098	0.281	0.038 .	-0.003 .	
0.346	0.037	0.025	0.277	-0.111	0.046	0.167	0.222 .	0.315	0.485

Table S2.7 (b) (continued)

(b)

			Ps. multicolor	*A. alluaudi*	*A. alluaudi*	*La.* sp. 'stone'	*Pa. chilotes*	*Pa. cyaneus*	*M. lutea*
			Sweya		Makobe				
			non-radiating lineages			*radiation lineage*			
non-radiating	Sweya	*A. alluaudi*	**0.730 ***						
		A. alluaudi	**0.619 ****	-0.044					
radiation lineage	Makobe	*La.* sp. 'stone'	0.323	0.746	1.000				
		Pa. chilotes	0.421	0.796	**1.000 ***	-0.179			
		Pa. cyaneus	0.513 .	0.811 .	**1.000 ****	-0.164	-0.088		
		M. lutea	0.078	0.802	1.000	0.000	0.125	0.236	
		M. mbipi	**0.396 ***	**0.729 ****	**0.754 ****	0.153	0.095	0.139	0.173
		N. gigas	0.287	0.788	**1.000 ***	-0.167	-0.009	0.036	0.167
		N. omnicaeruleus	**0.345 ***	**0.553 ***	**0.603 ****	-0.159	-0.130	-0.104	-0.039
		N. sp. 'unicuspid scraper'	**0.495 ***	**0.663 ****	**0.748 ****	-0.186	-0.155	-0.138	0.029
		N. rufocaudalis	0.373	0.818	**1.000 ***	-0.071	-0.083	0.000	-0.036
		P. sp. 'pink anal'	0.169	0.478	**0.384 ***	-0.214	-0.142	-0.060	-0.247
		P. pundamilia	0.520	**0.743 ****	**0.988 ****	-0.250	-0.117	-0.077	0.052
		P. nyererei	0.121	**0.354 ****	**0.375 ****	-0.133	-0.142	-0.113 .	-0.201
	Kissenda	*P.* sp. 'pundamilia-like'	-0.015	0.720	**0.750 ***	0.167	0.435	0.574	-0.250
		P. sp. 'nyererei-like'	**0.213 ***	**0.441 ***	**0.476 ****	0.338	0.250	0.281	0.166
		Pt. xenognathus	0.340	0.722	**1.000 ***	-0.167	-0.056	0.036	-0.208

Table S2.7 (b) (continued)

M. mbipi	*N. gigas*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*N. rufocaudalis*	*P.* sp. 'pink anal'	*P. pundamilia*	*P. nyererei*	*P.* sp. 'pundamilia-like'	*P.* sp. 'nyererei-like'
								Kissenda	
0.262									
0.002	-0.048								
0.038	-0.027	-0.037							
0.004	0.111	-0.130	-0.143						
0.075	-0.115	-0.004	0.056	-0.175					
0.244	-0.071	-0.033	-0.033	-0.048	-0.009				
-0.042	-0.076	-0.047	**-0.009 ***	-0.177	-0.063	-0.078			
0.257	0.389	0.210	0.349	0.315	-0.014	0.506	-0.027		
-0.002	0.357	0.123	0.182	0.149	0.153	0.302	**0.074 ***	0.072	
0.167	-0.037	-0.089	-0.064	-0.111	-0.171	-0.099	-0.155	0.074	0.224

Table S2.7 (c) (continued)

(c)

			Ps. multicolor	*A. alluaudi*	*Pa. cyaneus*	*M. mbipi*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*P.* sp. 'pink anal'	*P. pundamilia*	*P. nyererei*
			Sweya	Makobe							
			non-radiating		*radiation lineage*						
non-rad		*A. alluaudi*	**0.893 ****								
radiation lineage	Makobe	*Pa. cyaneus*	**0.688 ***	**0.924 ****							
		M. mbipi	**0.427 ***	**0.872 *****	**0.355 ***						
		N. omnicaeruleus	**0.362 ***	**0.763 *****	-0.010	0.013					
		N. sp. 'unicuspid scraper'	**0.543 *****	**0.875 *****	0.166	0.082	-0.016				
		P. sp. 'pink anal'	0.171	**0.700 ****	0.132	0.066	-0.030	0.126			
		P. pundamilia	**0.560 ***	**0.853 *****	0.073	0.276	-0.102	-0.003	0.009		
		P. nyererei	0.292 .	**0.795 *****	**0.154 ***	0.006	-0.025 .	**0.001 ***	-0.025	-0.015	
	Kissenda	*P.* sp. 'pundamilia-like'	**0.281 ***	**0.792 ****	0.440	**0.022 ***	0.129	0.149	0.098	0.281	**0.038 ***

Table S2.7 (d) (continued)

(d)

			Ps. multicolor	*A. alluaudi*	*Pa. cyaneus*	*M. mbipi*	*N. omnicaeruleus*	*N.* sp. 'unicuspid scraper'	*P.* sp. 'pink anal'	*P. pundamilia*	*P. nyererei*
			Sweya	Makobe							
			non-radiating		*radiation lineage*						
non-rad		*A. alluaudi*	**0.730 ****								
radiation lineage	Makobe	*Pa. cyaneus*	**0.513 ***	**0.811 ****							
		M. mbipi	**0.396 ***	**0.729 ****	0.139						
		N. omnicaeruleus	**0.345 ***	**0.553 ****	-0.104	0.002					
		N. sp. 'unicuspid scraper'	**0.495 ****	**0.663 ****	-0.138	0.038	-0.037				
		P. sp. 'pink anal'	0.169	**0.478 ****	-0.060	0.075	-0.004	0.056			
		P. pundamilia	0.520	**0.743 ****	-0.077	0.244	-0.033	-0.033	-0.009		
		P. nyererei	0.121 .	**0.354 ****	**-0.113 ***	-0.042	-0.047 .	**-0.009 ***	-0.063	-0.078	
	Kiss	*P.* sp. 'pundamilia-like'	**0.213 ***	**0.441 ****	0.281	-0.002 .	0.123	0.182	0.153	0.302	**0.074 ***


3

3

Variation in parasite infection between replicates of speciation in Lake Victoria cichlid fish

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4

4

Patterns of ectoparasite infection in wild-caught and lab-reared cichlid fish, and their hybrids, implicate extrinsic rather than intrinsic causes of species differences in infection

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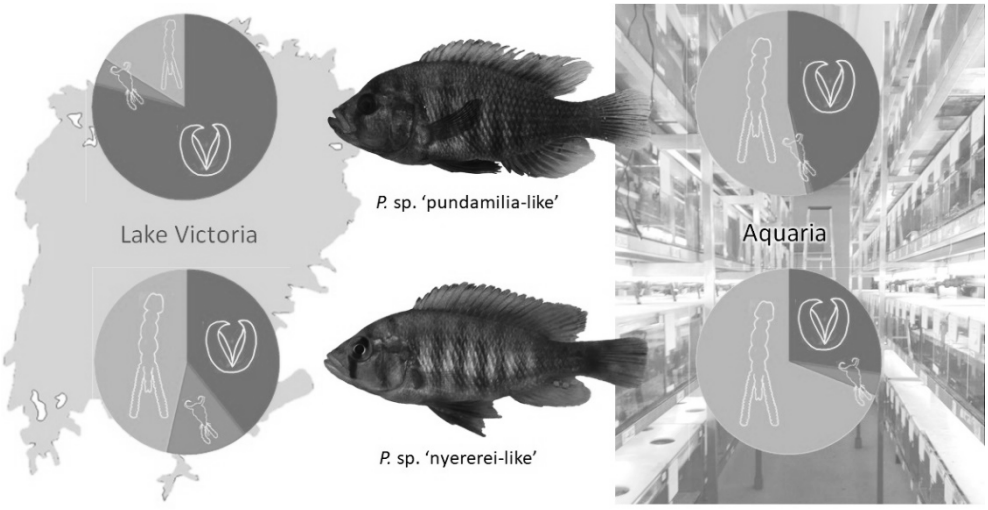
Lake Victoria
P. sp. 'pundamilia-like'
P. sp. 'nyererei-like'
Aquaria

ABSTRACT

Parasite-mediated selection may initiate or enhance differentiation between host populations that are exposed to different parasite infections. Variation in infection among populations may result from differences in host ecology (thereby exposure to certain parasites) and/or intrinsic immunological traits. Species of cichlid fish, even when recently diverged, often differ in parasite infection, but the contributions of intrinsic and extrinsic causes are unknown.

Here, we compare infection patterns between two closely related host species from Lake Victoria (genus *Pundamilia*), using wild-caught and first-generation laboratory-reared fish, as well as laboratory-reared hybrids. Three of the commonest ectoparasite species observed in the wild were also present in the laboratory populations. However, the infection differences between the host species as observed in the wild were not maintained in laboratory conditions. In addition, hybrids did not differ in infection from either parental species.

These findings suggest that the observed species differences in infection in the wild might be mainly driven by ecology-related effects (i.e. differential exposure), rather than by intrinsic species differences in immunological traits. Thus, while there is scope for parasite-mediated selection in *Pundamilia* in the wild, it has apparently not yet generated divergent evolutionary responses and may not enhance assortative mating among closely related species.

Keywords

parasite-mediated selection, diversification, host-parasite interaction, Lake Victoria, Cichlidae, Copepoda

4.1. INTRODUCTION

Parasites constitute a major source of ecological selection, as they impose fitness costs on their hosts (e.g. reduced growth, reproduction and survival, Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018), initiating coevolutionary dynamics of adaptation and counter-adaptation (Decaestecker et al., 2007). When different host populations encounter different parasites, they may engage in divergent co-evolutionary arms races. This may lead to host genetic divergence and eventually reproductive isolation (Hamilton & Zuk, 1982; Landry et al., 2001; Nosil et al., 2005; Maan et al., 2008; Eizaguirre et al., 2011), or it may strengthen differentiation once a certain level of reproductive isolation is already established through other mechanisms (Haldane, 1949; Price et al., 1986; Karvonen & Seehausen, 2012).

Heterogeneity in infection among populations or closely related species has been observed in a wide range of animal taxa (e.g. bivalves Coustau et al., 1991; fish Thomas et al., 1995; MacColl, 2009a; crustaceans Galipaud et al., 2017; reptiles Carbayo et al., 2018; mammals Boundenga et al., 2018). When host species differ in ecology (e.g. diet, habitat), they may be exposed to different parasites and adapt to these specific parasite threats by evolving resistance (which prevents or reduces infection) or tolerance (which reduces the fitness cost imposed by infection). Thus, variation among hosts in infection patterns is the result of host ecology, immune response and the interactions between them (Wolinska & King, 2009). The relative importance of such intrinsic and extrinsic factors in determining parasite infection patterns is often unknown. Controlled laboratory conditions offer the opportunity to experimentally standardize extrinsic factors, i.e. parasite exposure, to investigate the contribution of host intrinsic immunological properties to variation in infection.

Cichlid fish of the African Great Lakes form a well-studied example of adaptive radiation (Kornfield & Smith, 2000; Kocher, 2004; Seehausen, 2006). A large number of species has rapidly diverged through niche partitioning (Turner, 2007) resulting in a large diversity of macro-habitat, micro-habitat and trophic specializations (Sturmbauer & Meyer, 1992; Bouton et al., 1997; Genner et al., 1999). In several African cichlid lineages, species differences in ecology are associated with differences in the community composition of the parasites infecting them (Hablützel et al., 2017; Hayward et al., 2017; Karvonen et al., 2018), suggesting that variation in exposure contributes to variation in infection. Variation in immune response may have evolved as well: among closely related and sympatric cichlid species of Lake Malawi, differentiation in parasite community composition is correlated with differentiation at the MHC locus (Major Histocompatibility Complex, coding for proteins that recognize pathogens) (Blais et al., 2007).

Here, we investigate species differentiation in immune defense in two closely related Lake Victoria cichlids. To do so, we analysed the ectoparasite fauna of *Pundamilia* sp. 'pundamilia-like' and *Pundamilia* sp. 'nyererei-like', two weakly differentiated *Pundamilia* species from Lake Victoria, comparing wild-caught fish with the first-generation offspring of the same populations

raised in standardized laboratory conditions. In nature, these two species are sympatric but differ in their average depth distribution and diet. Previous studies in this species pair, as well as in closely related populations inhabiting other locations in Lake Victoria, revealed that they differ in parasite infection (Maan et al., 2008; Karvonen et al., 2018; **Gobbin et al., in prep.**). They mate assortatively, mediated by species-specific female preferences for male coloration (blue vs. red; Seehausen & van Alphen, 1998. In one population, females were shown to also express preferences for more brightly coloured males (Maan et al., 2004) and such males had lower parasite loads (Maan et al., 2006b), suggesting that there could be sexual selection for parasite resistance.

If species differences in infection are the result of genetically based differences in immune defence, then we expect to see the same differences in populations kept in standardized laboratory conditions, with uniform parasite exposure. If, on the other hand, species differences in infection are driven by heterogeneity in parasite exposure, then we expect such differences to disappear in laboratory conditions. We assessed infection patterns in *Pundamilia* sp. 'pundamilia-like' and *Pundamilia* sp. 'nyererei-like', as well as (in the laboratory) interspecific F1 hybrids. If parasite-mediated selection contributes to host reproductive isolation through selection against hybrids, then hybrids should have reduced resistance and will be more heavily infected than parental species. If, on the other hand, heterozygote advantage confers enhanced resistance, hybrids will be less infected and parasite-mediated selection could even hamper host divergence.

4.2. METHODS

4.2.1. Fish collection

Data on parasite infection, fish body size and water depth of wild-caught fish were retrieved from our previous field study (**Gobbin et al., in prep.**; **Table 4.1** and **Table S4.1**) based on a sample of male *Pundamilia* sp. 'pundamilia-like' (n=39) and *P.* sp. 'nyererei-like' (n=37; from now on referred to as P. pun and P. nye, respectively) collected in 2010 and in 2014 at Python Island in the Mwanza Gulf of Lake Victoria (-2.6237, 32.8567). Similar sympatric pairs co-occur at several rocky islands in the southeastern part of the lake (Meier et al., 2017a; Meier et al., 2018). Among islands, sympatric pairs vary in the level of reproductive isolation and in the extent of differentiation in ecological traits, such as water depth and diet (Seehausen, 1996a; Seehausen et al., 2008; Meier et al., 2017b; van Rijssel et al., 2018b; Wright et al., 2019). At Makobe Island, where these two sympatric species are strongly differentiated, they differ in their parasite abundances, in a way that is consistent with species differences in diet and microhabitat: P. pun harbour more intestinal nematodes and P. nye more gill copepods (Maan et al., 2008; Karvonen et al., 2018; **Gobbin et al., in prep.**). Less pronounced differences in parasite infection were found in populations inhabiting Kissenda and Python Islands (**Gobbin et al., in prep.**).

Live fish were collected in August 2010 and in October 2014 at the same location and brought to the aquarium facility of the Eawag Center for Ecology, Evolution and Biogeochemistry in Kastanienbaum (Switzerland), and moved to the University of Groningen (Netherlands) in September 2011 and in November 2014, respectively. The introduction of wild-caught fish in the aquaria coincidentally introduced some of their parasites as well.

First-generation laboratory-bred crosses (hybrid and non-hybrid) were created opportunistically, with 21 dams and 16 sires from the wild. Hybridization occurs with low frequency at Python Islands (Seehausen et al., 2008) and can be realised in the laboratory by housing females with heterospecific males. Thirty-eight F1 crosses (mother x father: 14 P. nye x P. nye; 12 P. pun x P. pun; 3 P. nye x P. pun; 9 P. pun x P. nye) resulted in a test population of 87 males from 38 families (30 P. pun, 31 P. nye, 26 hybrids; **Table S4.2**). Since our laboratory-bred individuals are produced from wild parents, we assume that the genetic diversity in the laboratory-bred population is not lower than in the wild. For the wild fish we only included males, because females are difficult to identify reliably in the field due to their cryptic coloration. Therefore to avoid confounding species differences with sex differences (Maan et al., 2006b) and to allow comparison we also included only males for the laboratory-reared fish.

Fish were maintained in recirculation aquariums (25 ± 1°C, 12L : 12D) and fed twice a day with a mixture of commercial cichlid flakes and pellets and defrosted frozen food (artemia, krill, spirulina, black and red mosquito larvae). The aquaria were divided into three light treatments, with separate circulation filters, used for studies on visual adaptation. In the wild, the two species are adapted to different visual environments, differing in opsin gene sequence and expression level (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019). In the laboratory, visual conditions were created with halogen light bulbs and coloured filters to mimic the natural light environments of both species at Python Island (detailed description in Maan et al., 2017; Wright et al., 2017). The “shallow light treatment” simulated the broad-spectrum light conditions of the shallow water habitat (0-5 m) of P. pun; the “deep light treatment” simulated the red-shifted light spectrum of the deep-water habitat (5-10 m) of P. nye. The resulting mismatch between the species’ visual adaptations and the visual environment was previously shown to affect survival: fish survived better under light conditions mimicking their natural habitat (Maan et al., 2017). Here, we explore whether this coincides with lower parasite loads. About half of the host individuals were reared and maintained in each condition. For the two non-hybrid groups, this implies that half of individuals were housed under experimental light mimicking their natural light environment (“natural light” 14 P. pun, 11 P. nye), while the other half were reared and maintained under experimental light mimicking the light condition of heterospecifics (“unnatural light” 15 P. pun, 18 P. nye; **Table S4.1**).

Table 4.1

Ectoparasite infection (% prevalence, abundance mean and range) of *Pundamilia* from Python Island, sampled in the wild and bred in the laboratory. Infection parameters of laboratory-bred fish are also reported according to the light treatment in which they were housed (natural or unnatural, except 3 fish housed in standard aquarium lighting).

	Host	N fish	*Lamproglena monodi*				*Ergasilus lamellifer*				Glochidia			
			%	median	mean	(min-max)	%	median	mean	(min-max)	%	median	mean	(min-max)
lab	*Pundamilia* sp. 'pundamilia-like'	30	70.0	4.0	5.13	(0-28)	10.0	0.0	0.10	(0-1)	10.0	0.0	4.40	(0-130)
	natural light	14	71.4	5.5	7.36	(0-28)	14.3	0.0	0.14	(0-1)	0.0	0.0	0.00	(0-0)
	unnatural light	15	66.7	2.0	3.33	(0-12)	6.7	0.0	0.07	(0-1)	20.0	0.0	8.80	(0-130)
	Pundamilia sp. 'nyererei-like'	31	51.6	2.0	4.09	(0-26)	12.9	0.0	0.23	(0-2)	6.5	0.0	1.61	(0-30)
	natural light	11	54.5	2.0	5.27	(0-26)	18.2	0.0	0.27	(0-2)	0.0	0.0	0.00	(0-0)
	unnatural light	18	50.0	1.0	3.72	(0-17)	11.1	0.0	0.22	(0-2)	11.1	0.0	2.78	(0-30)
	Pundamilia sp. 'hybrid'	26	61.5	1.5	3.50	(0-14)	15.4	0.0	0.27	(0-2)	3.8	0.0	0.12	(0-3)
wild	*Pundamilia* sp. 'pundamilia-like'	39	33.3	0.0	0.64	(0-4)	20.5	0.0	0.23	(0-2)	38.5	1.0	3.10	(0-15)
	Pundamilia sp. 'nyererei-like'	37	59.4	1.0	1.41	(0-11)	40.5	0.0	0.43	(0-2)	62.2	1.0	1.19	(0-6)

4.2.2. Parasite screening

To assess ectoparasite infection in laboratory-bred fish, we used individuals that naturally died (retrieved quickly after death to minimise the possibility that parasites would leave the host) or that were sacrificed for other experiments. Most fish (n=66) were preserved in 100% ethanol, while some were frozen (n=21). Fish were measured (SL standard length, BD body depth, to the nearest 0.1 mm) and weighed (to the nearest 0.1 g; **Table S4.1**). Gill arches were removed from the right side of each fish and then examined for ectoparasite infection under a dissecting stereoscope. All ectoparasites were identified following Paperna (1996) and counted. Analyses were conducted separately for prevalence (percentage of individuals infected of total examined host population) and abundance (mean number of parasites per individual of the examined host population) of each parasite taxon (**Table 4.1**). In addition to parasite counts, we also assessed the proportion of parasitic copepods carrying egg clutches, as a proxy of copepod reproductive activity, which may indicate how well the parasites thrive on a given host species (Paperna, 1996).

4.2.3. Data analysis

To investigate differences in ectoparasite community composition between host groups we performed one-way analysis of similarities based on the zero-adjusted Bray-Curtis distances of parasite abundance data (ANOSIM, 9999 permutations, PAST 3.18, Hammer et al., 2001). To compare infection abundance and prevalence of each ectoparasite taxon separately, we performed generalized linear models using the *lmer* function in *lme4* package (Bates et al., 2015) in R (R Core Team, 2019), using binomial distribution for the former and Poisson distribution for the latter. Since overdispersion was detected in parasite abundance models, we corrected the standard errors using a quasipoisson model (Zuur et al., 2009). Additional details are given below.

We investigated differences in ectoparasite community composition and in infection levels between groups, in the wild and in the laboratory, as indicated above. Fixed effects included host species, wild/lab status, fish length (SL; to account for species differences in size, as P. pun is larger than P. nye and laboratory-bred fish tend to be larger than wild ones, **Fig. S4.1**) and all possible interactions between them, as well as the year of fish collection and circumstances of death (naturally died or sacrificed). We determined the significance of fixed effects by likelihood ratio tests (LRT) to select the Minimum Adequate Model (MAM) via the *drop1* function in the *stats* package. Least square means was used to compare infection between host species in the wild and in the laboratory (*lsmeans* in the *emmeans* package, Lenth, 2019).

Infection levels in hybrids

A potential hybrid (dis)advantage in parasite infection was investigated by comparing infections (parasite community composition, prevalence and abundance) of laboratory-bred interspecific

F1 hybrids with F1 laboratory-bred P. pun and P. nye, as indicated above. Fixed effects included host group (P. pun, P. nye, hybrids), fish individual age, fish length (SL) and circumstances of death (naturally died or sacrificed), as well as the following interactions: between host group and all other variables, between age and SL, between age and circumstances of death, between circumstances of death and SL, between host group, circumstances of death and SL. Random effects included filter system and family to account for separate water circulating systems and for shared parentage among fish, respectively. We selected the MAM and used least square means for comparisons, as above.

Effect of light treatments on infection

We investigated whether parasite infection differed between individuals reared and maintained in different light treatments (shallow vs. deep and natural vs. unnatural), as indicated above. First, we assessed a possible overall effect of the light treatment (shallow vs. deep, irrespective of the host species' natural conditions). Second, we assessed a possible effect of light-matching conditions (natural vs. unnatural). Fixed effects included host species (P. pun, P. nye, hybrids), fish individual age, length (SL), circumstances of death (naturally died or sacrificed), light condition (shallow vs. deep and natural vs. unnatural). The following interactions were also included: between host species and all other variables, between light treatment and all other variables, between circumstances of death and all other variables, between age and SL, between host species, SL and light treatment as well as between host species, SL and circumstances of death. Random effects included family to account for shared parentage among fish. We selected the MAM as mentioned above, then we tested the MAM against a model including the light treatment parameter (shallow vs. deep and natural vs. unnatural visual environment).

Reproductive activity of copepods

Using generalized linear models (*glm* function in the *stats* package), we compared the proportion of copepods carrying egg clutches between infected individuals of wild-caught and laboratory-bred hosts of both parental species. Fixed effects included host species, wild/lab status, their interaction, and fish individual length. We determined the significance of fixed effects by LRT and we used least square means as post-hoc to obtain parameter estimates.

The same procedure was applied to test for variation in reproductive activity of copepods among infected laboratory-bred host groups (P. pun, P. nye, interspecific hybrids). Fixed effects included host species and fish individual length.

4.3. RESULTS

Four ectoparasite taxa were observed in the laboratory: *Lamproglena monodi* Capart, 1944, an unidentified *Lamproglena* species (Copepoda: Cyclopoida), *Ergasilus lamellifer* Fryer, 1961 (Copepoda: Poecilostomatoida) and glochidia mussel larvae (Bivalvia: Unionoidea). These were also observed in *Pundamilia* sampled from the wild, except the unidentified *Lamproglena* (which was observed in only one laboratory-bred hybrid individual, and excluded from statistical analysis). The monogenean *Cichlidogyrus* spp. Paperna, 1960, which is abundant in wild Lake Victoria cichlids including *Pundamilia* spp., was absent from our aquarium facility. Infection levels are reported in **Table 4.1**.

Both copepods can infect a relatively wide range of cichlid species (Scholz et al., 2018) and have a fully limnetic direct life cycle with several planktonic non-parasitic stages (Paperna, 1996). Only adult females of *E. lamellifer* are parasites of fish (mainly cichlids), whereas both of the final development stage, and adult females of *L. monodi* are parasites of African cichlids. The mollusc may belong to Unioniformes, which infect the gills of cichlids at larval stages, displaying different degrees of host specificity (Wächtler et al., 2001; Haag & Warren, 2003), while juveniles and adults are free-living.

We did not observe overall differences in variance between laboratory-bred and wild populations (**Table S4.3**). Overall, infection abundance of *L. monodi* was higher in laboratory conditions (pooling P. pun and P. nye, and excluding hybrids) than in the wild (mean abundance ± SE laboratory 4.61±0.79 vs. wild 1.01±0.19; **Table 4.2b**), whereas prevalence did not differ (60.9% vs. 46.4%). On the contrary, *E. lamellifer* was more prevalent and more abundant in the wild than in the laboratory (prevalence 30.3% vs. 11.5%; abundance 0.34±0.06 vs. 0.16±0.06). Glochidia were more prevalent in the wild (60.5% vs. 8.2%) but had similar abundances in wild and laboratory conditions (abundance 2.17±0.38 vs. 2.98±2.19). The range of intensities of glochidia infection (i.e. number of parasites in infected individuals of the examined host population) was narrower in the wild than in the laboratory (1-15±0.54 vs. 1-130±24.06).

4.3.1. Species differences in infection

In the wild-caught fish, the ectoparasite community composition differed between *Pundamilia* species: P. nye had more *L. monodi* and *E. lamellifer* ($p<0.01$) and tended to have higher prevalence of glochidia ($p=0.053$). In the laboratory populations, there was no difference in ectoparasite community composition between the two species (**Fig. 4.1**, **Table 4.2a**).

We then tested species differences in infection for each ectoparasite taxon separately. After accounting for the differences in infection between wild and laboratory conditions (see above), we found that the two species differed in infection in the wild but not in laboratory conditions

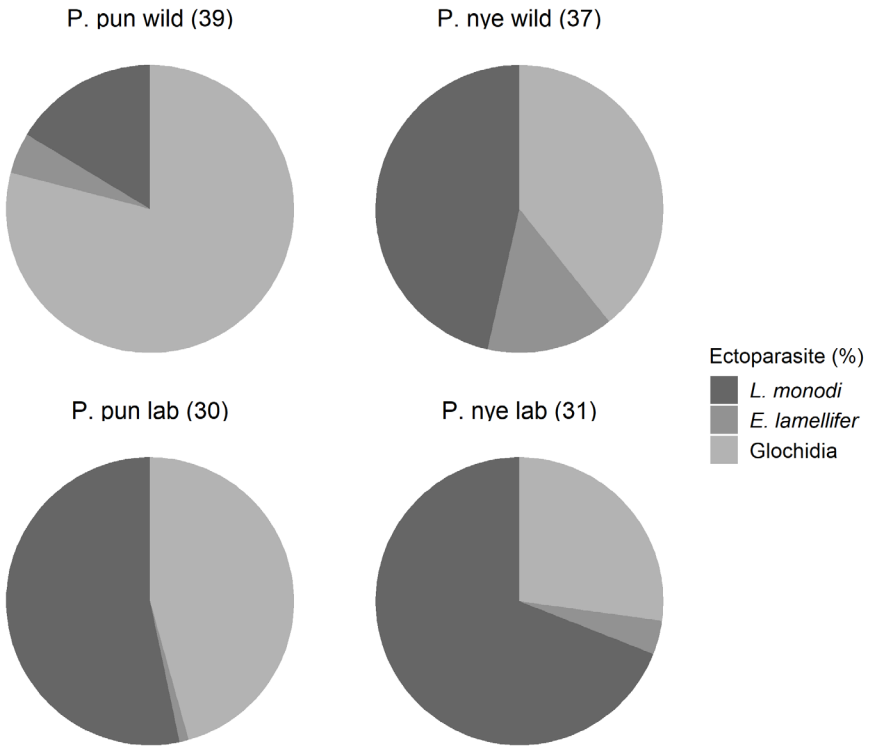

Figure 4.1
Ectoparasite community composition of wild and laboratory-bred *Pundamilia.* sp. 'pundamilia-like' (P. pun wild, P. pun lab) and *Pundamilia* sp. 'nyererei-like' (P. nye wild, P. nye lab). Charts include the three ectoparasite taxa that were present in both wild-caught and laboratory-bred fish (*Lamproglena monodi, Ergasilus lamellifer* and glochidia (mollusc larvae)). Species differences were significant in the wild (P. nye had more *L. monodi* and *E. lamellifer*), but not in the laboratory.

(**Fig. 4.2**, **Table 4.2c**). The difference between laboratory and field was significant for the species differences in infection with both copepods (i.e. significant interaction between species and wild/lab status for copepod prevalence and abundance, **Table 4.2bc**). The species differences in prevalence and abundance of *E. lamellifer* and glochidia did not significantly differ between wild and laboratory-reared fish. Post hoc analysis showed that in the wild, P. pun and P. nye differed in infection with *L. monodi* and of *E. lamellifer.* Both prevalence and abundance, of both copepods, were significantly higher in P. nye than in P. pun (prevalence *L. monodi* 59.46% vs. 33.33%; prevalence *E. lamellifer* 40.54% vs. 20.51%; mean abundance *L. monodi* 1.41 vs. 0.64; mean abundance *E. lamellifer* 0.43 vs. 0.23). In the lab, the two species did not differ in prevalence nor in abundance of any ectoparasite (**Fig. 4.2**, **Table 4.2**). Prevalence and abundance

of glochidia did not differ between the species, in either field or laboratory (in the wild, glochidia tended to be more prevalent in P. nye than in P. pun, p=0.053). The loss of the species difference in infection of *L. monodi* in laboratory conditions was largely due to an increased infection in laboratory-bred P. pun individuals in comparison to their wild-caught counterparts. For *E. lamellifer,* it was largely due to a decreased infection in laboratory-bred P. nye in comparison to their wild-caught counterparts.

Overall (pooling P. pun and P. nye from the wild and from the lab), the prevalence and abundance of *L. monodi* (but not of *E. lamellifer* and of glochidia) increased with fish length (**Table 4.2b**). However, wild-caught P. nye had more copepods than expected based on their size, while in laboratory conditions, this disproportionate infection level disappeared.

4.3.2. Infection levels in hybrids

The ectoparasite community composition did not significantly differ between laboratory-bred parental species and their hybrids (R_2=-0.011, p=0.704; **Table 4.3a**). The average dissimilarity of the ectoparasite community was as large between hybrids and each parental species (average dissimilarity hybrids vs. P. pun 49.76; hybrids vs. P. nye 48.62) as it was between the two parental species (P. pun vs. P. nye 51.57).

When testing each ectoparasite taxon separately, infection prevalence nor abundance differed between hybrids and either parental species (**Fig. 4.2**, **Table 4.3b**). Variation in prevalence (but not abundance) of *L. monodi* in laboratory-bred fish was associated with fish length: larger individuals were more often infected (LRT_1=15.38, p<0.001). Variation in abundance (but not prevalence) of the other two parasites, *E. lamellifer* and glochidia, were not associated with any of the assessed variables (host group, fish individual length, age; **Table 4.3b**).

We had expected higher infection levels in fish that died naturally (as they might be in poor health) compared to sacrificed fish, but we did not observe this. Laboratory fish that were sacrificed had a higher prevalence and abundance of *L. monodi* than those that died naturally. This cannot be explained by fish age or size. The effect of fish age on prevalence of *E. lamellifer* differed between circumstances of death: sacrificed fish were more likely to be infected when they were older, while prevalence and age were not associated in naturally died fish.

Table 4.2
Variation in infection among *Pundamilia* sampled at Python Island (wild) and their laboratory-bred counterparts (lab). **(a)** Differences in ectoparasite community composition, based on zero-adjusted Bray-Curtis distances (ANOSIM, 9999 permutations). Upper diagonal reports p-values (Benjamini-Hochberg corrected), lower diagonal ANOSIM R-values (significant differences in boldscript). **(b)** Variation in prevalence and abundance of individual ectoparasite taxa. The Minimum Adequate Model (MAM, shown in black) was established by stepwise removal of non-significant variables (not shown). The effect of host species and wild/lab status combined was also assessed separately in a reduced model including these parameters (shown in grey). **(c)** post hoc comparison (least square means) between the two host species in the lab and in the wild. *SL* fish standard length, *wildlab* wild-caught or laboratory-bred fish, *circ death* circumstances of death.

		lab		wild	
(a)		P. pun	P. nye	P. pun	P. nye
lab	P. pun		0.316	<0.001	<0.001
	P. nye	0.001		<0.001	<0.001
wild	P. pun	**0.240**	**0.209**		0.006
	P. nye	**0.256**	**0.163**	**0.078**	

		prevalence					**abundance**				
(b)		**fixed factor**	**Chisq**	**df**	**p**		**fixed factor**	**Chisq**	**df**	**p**	
Lamproglena monodi		species	5.87	1	0.015	*	wildlab	4.22	1	<0.001	***
		SL	10.05	1	0.002	**	SL	9.27	1	0.002	**
		circ death	8.91	1	0.003	**	circ death	21.69	1	<0.001	***
		species:wildlab	19.53	2	<0.001	***	species:wildlab	8.33	2	0.016	*
		species:SL	1.56	1	0.033	*					
		species:wildlab	10.42	3	0.015	*	species:wildlab	38.06	3	<0.001	***
		species:wildlab:SL	14.41	4	0.006	**	species:wildlab:SL	11.36	4	0.023	*
Ergasilus lamellifer		species	8.23	1	0.004	**	species	8.60	1	0.003	**
		wildlab	8.54	1	0.003	**	wildlab	6.24	1	0.012	*
		SL	6.33	1	0.012	*	SL	6.31	1	0.012	*
		circ death	4.01	1	0.045	*	circ death	5.45	1	0.01956	*
		species:wildlab	11.87	3	0.008	**	species:wildlab	7.95	3	0.047	*
		species:wildlab:SL	12.29	4	0.015	*	species:wildlab:SL	7.66	4	0.105	
Glochidia		SL	4.74	1	0.030	*	wildlab	5.63	1	0.018	*
		year	74.33	1	<0.001	***	year	5.43	1	0.020	*
							circ death	10.20	1	0.001	**
							species:wildlab	10.48	2	0.005	**
							species:SL	9.15	2	0.010	*
							wildlab:SL	4.05	1	0.044	*
							species:wildlab:SL	6.73	1	0.009	**
		species:wildlab	42.25	3	<0.001	***	species:wildlab	2.77	3	0.429	
		species:wildlab:SL	10.06	4	0.039	*	species:wildlab:SL	8.90	4	0.064	.

Table 4.2 (continued)

		prevalence					abundance			
(c)		**estimate**	**z**	**p**			**estimate**	**z**	**p**	
Lamproglena monodi	wild: P < N	-2.90	-3.81	<0.001	***	wild: P < N	-1.49	-2.71	0.007	**
	lab: N vs. P	1.06	1.42	0.156		lab: N vs. P	-0.05	-0.17	0.863	
	P: wild < lab	-2.41	-3.42	0.001	***	P: wild < lab	-2.18	-5.02	<0.001	***
	N: wild vs. lab	1.54	1.90	0.057	.	N: wild < lab	-0.74	-1.96	0.050	*
Ergasilus lamellifer	wild: P < N	-2.29	-2.84	0.005	**	wild: P < N	-1.53	-2.61	0.009	**
	lab: P vs. N	-0.80	-0.83	0.405		lab: P vs. N	-1.07	-1.45	0.148	
	P: wild vs. lab	0.84	1.08	0.279		P: wild vs. lab	0.75	1.16	0.245	
	N: wild > lab	2.33	2.81	0.005	**	N: wild > lab	1.20	2.17	0.030	*
Glochidia	wild: P < N	-1.44	-1.93	0.053	.	wild: P vs. N	-0.11	-0.06	0.949	
	lab: P vs. N	-0.60	-0.58	0.560		lab: P vs. N	15.90	0.47	0.642	
	P: wild vs. lab	0.06	0.06	0.952		P: wild > lab	-4.43	-2.15	0.032	*
	N: wild vs. lab	0.90	0.81	0.420		N: wild vs. lab	11.58	0.34	0.735	

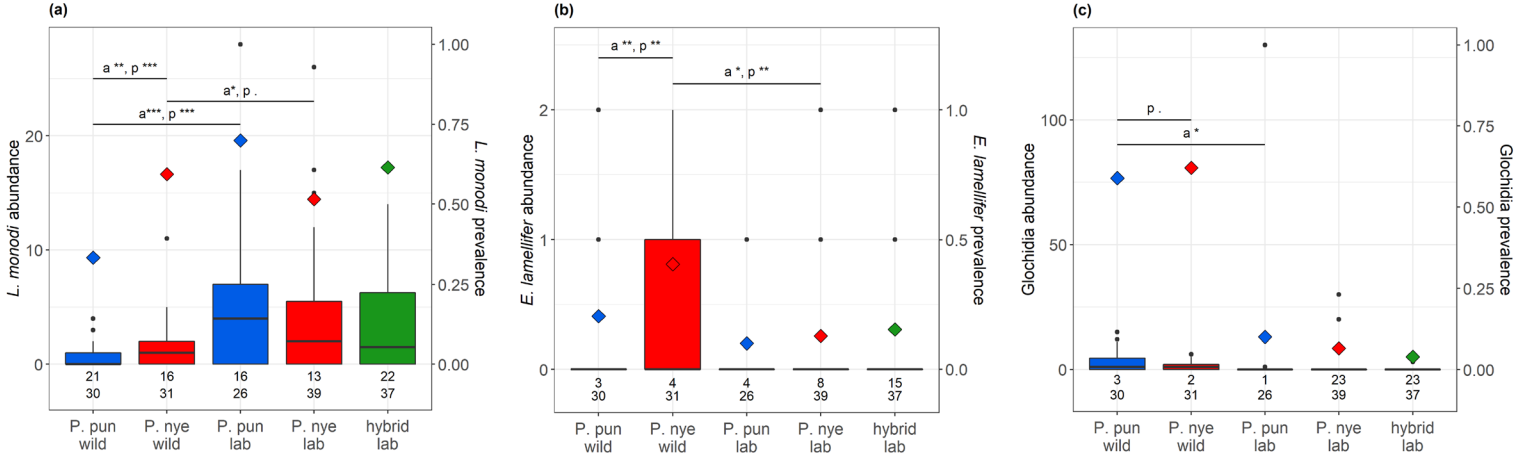

Figure 4.2
Ectoparasite abundance (boxes) and prevalence (diamonds) of wild and first-generation laboratory-bred *Pundamilia.* sp. 'pundamilia-like' (P. pun wild, P. pun lab) and *P.* sp. 'nyererei-like' (P. nye wild, P. nye lab) as well as their first-generation laboratory-bred hybrids (hybrid lab). **(a)** *Lamproglena monodi*, **(b)** *Ergasilus lamellifer*, **(c)** glochidia. Numbers of infected fish individuals per species (upper row) and total sample size per species (lower row) are reported. Asterisks indicate significance level for abundance *(a)* and prevalence *(p)*. Copepod infection levels differed between the two host species in the wild, but not in laboratory conditions. Glochidia infection did not differ between species in either wild-caught or laboratory-bred populations (in the wild, P. pun tended to have a higher prevalence of glochidia than P. nye, p=0.053). Infection levels of hybrids did not differ from those of parental species, for any of the parasites. Black symbols are outliers.

4.3.3. Light treatments and infection

The ectoparasite community composition did not differ between the two light treatments (deep vs. shallow light regimes, R_1=-0.018, p=0.757). It also did not differ between light-matching conditions (natural vs. unnatural light; pooling both species: R_1=0.007, p=0.309; for each species separately: P. pun R_1=0.019, p=0.227; P. nye R_1=-0.041, p=0.747, **Table S4.5a**).

When considering individual ectoparasite taxa, there were no overall differences between deep and shallow light treatments in infection prevalence or abundance (**Table S4.4**). However, fish reared and maintained under natural light conditions (pooling both host species) had lower prevalence of glochidia than fish housed in unnatural light conditions (**Fig. 4.3**, **Table S4.5b**). The infection prevalence and abundance of the other ectoparasites did not differ between fish in natural and unnatural light conditions. When looking at the two host species separately, we found no significant differences in the prevalence or abundance between natural and unnatural light (**Table S4.5b**). *Pundamilia* sp. 'pundamilia-like' tended to have a higher prevalence of glochidia when housed in the unnatural light condition (**Table S4.5c**).

4.3.4. Reproductive activity of copepods

Of 316 individuals of *L. monodi* (wild and laboratory combined, hybrids excluded), 73.7% carried egg clutches. The proportion of *L. monodi* carrying egg clutches did not differ in any of the comparisons made (between host species, between wild and laboratory conditions, between hybrids and parentals; **Table S4.6a-b**, **Fig. S4.2**). Of 26 individuals of *E. lamellifer*, 51.8% carried egg clutches. The proportion of *E. lamellifer* carrying egg clutches did not differ between host species, nor between hybrids and parentals. It did differ between wild and laboratory conditions, as none of the few representatives of *E. lamellifer* in the laboratory (17 in total) had egg clutches. The abundance of conspecifics was not correlated with the proportion of egg-carrying individuals. In the field, *L. monodi* and *E. lamellifer* were more likely to carry egg clutches in larger fish (**Table S4.6a**).

Table 4.3
Differences in infection between F1 laboratory-bred *P.* sp. 'pundamilia-like' (P. pun), *P.* sp. 'nyererei-like' (P. nye) and their F1 hybrids (hybrid) **(a)** Differences in ectoparasite community composition, based on zero-adjusted Bray-Curtis distances (ANOSIM, 9999 permutations). Upper diagonal reports p-values (Benjamini-Hochberg corrected), lower diagonal R-values. **(b)** Variation in prevalence and abundance of individual ectoparasite taxa. The Minimum Adequate Model (MAM) was established by stepwise removal of non-significant variables (not shown). **(c)** post hoc comparison (least square means) between the two host species in the lab and in the wild. *SL* fish standard length, *circ death* circumstances of death.

(a)	P. pun	P. nye	hybrid
P. pun		0.320	0.687
P. nye	0.001		0.888
hybrid	-0.015	-0.023	

		prevalence					**abundance**			
(b)	**fixed effect**	**Chisq**	**df**	**p**		**fixed effect**	**Chisq**	**df**	**p**	
Lamproglena monodi	SL	15.38	1	<0.001	***	age	12.63	1	<0.001	***
	circ death	6.49	1	0.011	*	circ death	14.48	1	<0.001	***
Ergasilus lamellifer	age:circdeath	6.73	2	0.035	*	1				
Glochidia	1					1				

		prevalence			**abundance**		
(c)	**comparison**	**estimate**	**t**	**p**	**estimate**	**t**	**p**
L. monodi	P. pun vs. P. nye	0.08	0.59	0.828	1.26	0.83	0.690
	P. pun vs. hybrid	-0.04	-0.28	0.957	1.94	1.13	0.508
	P. nye vs. hybrid	-0.13	-0.86	0.671	0.68	0.41	0.910
E. lamellifer	P. pun vs. P. nye	0.00	-0.03	1.000	-0.13	-0.95	0.618
	P. pun vs. hybrid	0.00	0.00	1.000	-0.08	-0.52	0.861
	P. nye vs. hybrid	0.00	0.02	1.000	0.05	0.34	0.938
Glochidia	P. pun vs. P. nye	-0.03	0.39	0.921	2.73	0.68	0.780
	P. pun vs. hybrid	0.01	0.10	0.995	4.26	0.95	0.616
	P. nye vs. hybrid	0.04	0.46	0.891	1.53	0.35	0.934

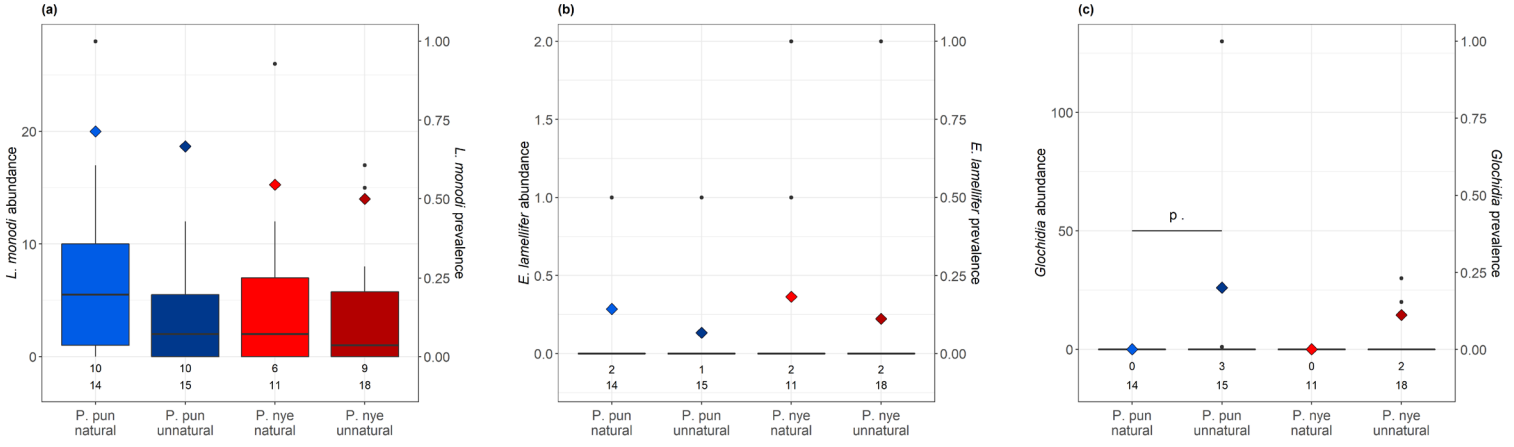

Figure 4.3
Ectoparasite abundance (boxes) and prevalence (diamonds) of laboratory-bred *Pundamilia* sp. 'pundamilia-like' (P. pun), *P.* sp. 'nyererei-like' (P. nye) raised in natural or unnatural light conditions. **(a)** *Lamproglena monodi*, **(b)** *Ergasilus lamellifer*, **(c)** glochidia. Numbers of infected individuals per species (upper row) and total sample size per species (lower row) are reported. Asterisks indicate significance level for abundance *(a)* and prevalence *(p)*. Infection levels did not differ between natural and unnatural light conditions (except for glochidia, that was more prevalent in the unnatural light conditions: statistical trend in P. pun, significant when pooling both host species). Black symbols are outliers.

4.4. DISCUSSION

Comparison of the ectoparasite infection patterns between wild-caught hosts and their laboratory-bred counterparts with uniform exposure, revealed infection divergence between P. pun and P. nye in the wild, but not in laboratory conditions. This indicates that the contribution of ecology-related factors (exposure) to infection variation might be larger than that of intrinsic factors related to parasite defence (i.e. genetically based variation in susceptibility). Comparison of ectoparasite prevalence, abundance and community composition between F1 hybrids and the two parental species in the laboratory showed no infection differences, contrary to the hypothesis that parasite-mediated selection promotes assortative mating in this species pair.

4.4.1. Species differences in infection disappear when exposure is homogeneous

In our previous studies, we found that populations of *Pundamilia* with intermediate differentiation, inhabiting Kissenda and Python Islands, showed some infection divergence (**Gobbin et al., in prep.**). In the wild, P. nye are more frequently infected, and in higher numbers, with *L. monodi* and *E. lamellifer* than P. pun (Maan et al., 2008; Karvonen et al., 2018; **Gobbin et al., 2020b**). Here, we report that these differences were absent in fish raised in the laboratory, where the expression of species-specific depth and diet preferences is impossible due to uniform housing conditions. This suggests that species differences in infection in wild *Pundamilia* might be primarily driven by differences in ecology-related traits, rather than by intrinsic differences in immunity or susceptibility. A large contribution of ecological factors to parasite infection has previously been documented in threespine stickleback of Canadian lakes, where individual foraging differences resulted in variation in infection in the wild (Stutz et al., 2014). The lack of consistency in species differences in infection between wild-caught and laboratory-bred hosts was also observed in threespine stickleback of Scottish lakes, in which the expression of immune genes of wild fish differed from that of laboratory-reared counterparts (Robertson et al., 2016).

While parasites might represent a major diversifying selective force in species divergence in nature, our findings are inconsistent with a role of parasite-mediated selection in the divergence of P. pun and P. nye at Python Island. Possibly, the divergence of these species is so recent, that species differences in ectoparasite-related immunity have not yet evolved. Python was colonized by *P. pundamilia* only a few thousand years ago, later followed by *P. nyererei* with which it admixed (Meier et al., 2017b; Meier et al., 2018). This hybrid population later speciated into a sympatric species pair of blue and red *Pundamilia* that resemble the original species currently occurring at Makobe Island, 31 km north of Python.

Infection differences between host species may become apparent only at a certain level of exposure. For *E. lamellifer* and glochidia, which had lower prevalence and abundance in the laboratory than in the field, this could contribute to the loss of species differences in infection in

the laboratory. For *L. monodi* however, which is the ectoparasite that differs most strongly between P. pun and P. nye in the wild, prevalence and abundance were comparable between laboratory and field.

Not all macroparasites observed in the wild were also present in the laboratory populations: intestinal nematodes and gill monogeneans were absent in the aquaria. Thus, laboratory fish experience only a fraction of the parasite threat of that in nature, which may influence how fish respond to infection. For example, in some wild populations of *Pundamilia*, nematodes contribute significantly to the species differences in infection profile between blue and red fish (Maan et al., 2008; Karvonen et al., 2018; **Gobbin et al., in prep.**). If this is due to genetic differences in susceptibility and if nematode infection levels influence an individual's response to other parasites, this may affect the species difference in ectoparasite infection as well. Since nematodes are absent in the laboratory, this effect cannot occur in the lab, implying that we cannot rule out genetically based species differences in susceptibility based on the findings presented here.

Parasites are generally expected to adapt to locally abundant host populations (especially parasite species with high host specificity; Lively, 1989; Lively & Dybdahl, 2000; Lajeunesse & Forbes, 2002). In the laboratory, this process could have caused a weakening of possible differences in infection between host species over time. It would also lead to a general increase of the infection rate with time. We do indeed observe an increase in infection rate, but no weakening of species differences over time (**Fig. S4.3**), suggesting that the observed similarity in infection among host species cannot be explained by parasites that have adapted to the laboratory conditions and host availability.

4.4.2. Hybrid equality rather than hybrid disadvantage

In laboratory conditions, hybrids did not differ from either parental species in ectoparasite infection prevalence, abundance, community composition nor in the proportion of copepods carrying egg clutches. This suggests that parasites do not promote reproductive isolation between P. pun and P. nye, contrary to a parasite-mediated diversification scenario. Our results are in line with previous research on the same study system: no intrinsic fitness reduction was observed in *Pundamilia* hybrids originating from Python-Island parents, for multiple traits (fecundity, fertility, sex ratio, growth rate, van der Sluijs et al., 2008b; and survival, Maan et al., 2017). Yet, hybrids are rarely observed in the wild (Seehausen et al., 2008). This indicates some selection against hybrids, as supported by mate choice studies: non-hybrid females prefer to mate with conspecific males and avoid both heterospecific and hybrid males (Seehausen & van Alphen, 1998; Stelkens et al., 2008; Selz et al., 2014). The absence of parasite-mediated hybrid disadvantage, as observed in the present study, suggests that parasites do not contribute to species-assortative mating, and hence additional drivers should be involved. In particular, species-assortative mating might be promoted by divergent selection on visual system properties (Seehausen et al., 2008; Maan et al., 2017).

To fully understand the potential for parasite-mediated selection against hybrids of *Pundamilia*, future research should include additional generations of hybrids and backcrosses, as these may differ in heritable parasite resistance. For example, F1 hybrids of European house mouse were found to be more resistant than parental species (Moulia et al., 1996), whereas hybrid backcrosses were more susceptible (Moulia et al., 1991). In African cichlids, male attractiveness and survival are lower in F2 hybrids compared to F1 hybrids (Svensson et al., 2011; Stelkens et al., 2015).

Although these findings suggest that parasites do not promote assortative mating in *Pundamilia,* it might be that in the wild selection on parasite resistance is different from the aquarium environment. Indeed, hybrid fitness in sticklebacks differ between laboratory and field conditions (Hatfield & Schluter, 1999), which suggests that the hybrid disadvantage observed in some species in the field may result from ecological components, including a diverse parasite community, rather than from intrinsic species traits (e.g. genetic incompatibilities).

4.4.3. Infection and light (mis)match in laboratory-bred *Pundamilia*

Parasite infection did not differ between light treatments (deep vs. shallow), nor between natural and unnatural light conditions – except perhaps for glochidia, the second most abundant ectoparasite in our aquarium facility. Glochidia were more prevalent (but not more abundant) in fish housed in unnatural light conditions. This is in line with the earlier observation that *Pundamilia* have lower survival when reared in unnatural visual conditions, compared to conspecifics reared in their natural light environment (Maan et al., 2017). Unnatural light conditions can be stressful to fish (Migaud et al., 2007), increase aggression (Carvalho et al., 2013) and decrease foraging performance (Rick et al., 2012). This could influence the probability of infection. However, infection parameters for the other two parasites did not differ between light conditions, making it unlikely that parasites contribute substantially to the differential mortality observed by Maan et al. (2017). This is consistent with the lower parasite abundance in naturally died fish compared to the sacrificed ones. We do not know how to interpret the difference in infection abundance between naturally died and sacrificed fish, but it is very unlikely that parasites have left the host because we only considered freshly died individuals. Since fish that had naturally died were older, we can speculate that they have survived for a long time because they are in good physical condition and therefore they have a low parasite load.

4.4.4. Reproductive activity of copepods

Both copepod species maintained viable populations in our laboratory, as they were present in the fish for at least 8 years after being introduced from the wild. Copepod reproductive activity (measured as the proportion of individuals carrying egg clutches) did not differ between the two host species in the wild, nor between laboratory-bred populations (P. pun, P. nye, interspecific hybrids). This suggests that differences in host ecology have little effect on the reproductive activity of copepods. In the laboratory, we observed reproductive activity only in *L. monodi*,

while representatives of *E. lamellifer* were never observed carrying egg clutches. Possibly, the low abundance of *E. lamellifer* (0-2 individuals per host) decreases mating opportunities. In addition, specific aspects of *E. lamellifer* life history may reduce the chance of detecting individuals carrying egg clutches (i.e. short egg incubation time, fewer reproductive phases per year, periods without ovigerous females; Paperna & Zwerner, 1976). Alternatively, egg clutches might occasionally detach from the body (but we do not observe that during manipulation). *Lamproglena monodi* was more abundant (up to 28 individuals per host) and showed equal reproductive activity across host species and laboratory populations. This may indicate that this is a generalist parasite, in line with its presence in many other cichlid species (Abdel-Gaber et al., 2017; Karvonen et al., 2018; Scholz et al., 2018; **Gobbin et al., 2020b**).

4.5. CONCLUSION

Infection differences between P. pun and P. nye were observed in the wild but not in laboratory conditions with uniform parasite exposure. This suggests that ecological-related traits affecting parasite exposure – rather than intrinsic differences in immunity or susceptibility – might explain the species differences in infection in the wild. Consistent with this, laboratory-bred hybrids did not differ in infection from either parental species. Together, these findings suggest that P. pun and P. nye may not differ in genetically based parasite resistance, despite the opportunity for parasite-mediated divergent selection in nature.

4.6. ACKNOWLEDGEMENTS

This research was funded by the Swiss National Science Foundation and the University of Groningen (Ubbo Emmius Programme).

4.7. SUPPLEMENTARY MATERIAL

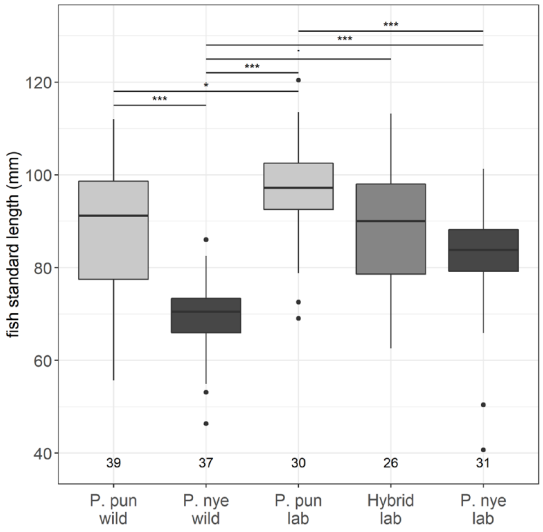

Figure S4.1
Fish size (standard length) of wild-caught and laboratory-bred *Pundamilia* sp. 'pundamilia-like' (P. pun wild, P. pun lab), *P.* sp. 'nyererei-like' (P. nye wild, P. nye lab) and their interspecific hybrids (lab only). Asterisks indicate significance levels; numbers indicate sample size (numbers of host individuals). Black symbols are outliers.

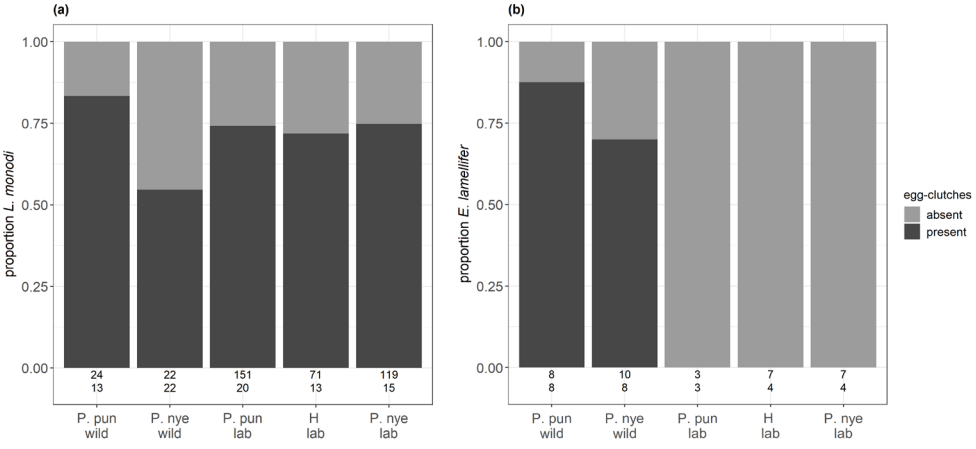

Figure S4.2
Proportion of copepods carrying egg clutches in wild and laboratory-bred *Pundamilia* sp. 'pundamilia-like' (P. pun lab), *P.* sp. 'nyererei-like' (P. nye lab) and their hybrids (H lab). **(a)** *Lamproglena monodi*, **(b)** *Ergasilus lamellifer*. No differences were observed between groups, except the higher proportion of egg-carrying *E. lamellifer* in the wild compared to the lab. Numbers indicate sample size of parasites (upper row) and sample size of infected fish (lower row).

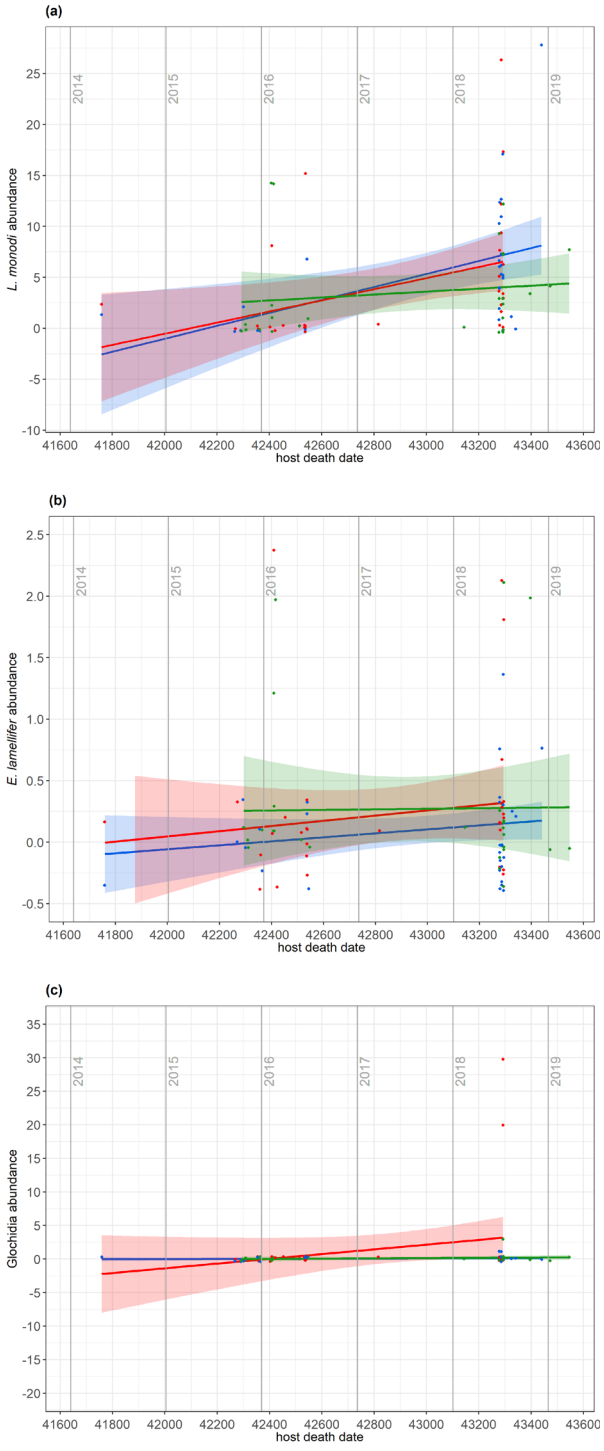

Figure S4.3
The infection abundance of **(a)** *Lamproglena monodi*, **(b)** *Ergasilus lamellifer*, and **(c)** glochidia increased with time elapsed since fish and parasites were introduced into the aquaria, but host species differences in infection did not decrease with time. Solid lines do not indicate a significant association.

Table S4.1
Characteristics of *Pundamilia* sampled at Python Island (wild) and their laboratory-bred counterparts (lab). SL standard length (mm), weight (g), age (days, data available for laboratory-bred fish only). Fish characteristics of laboratory-bred fish are also reported according to the light treatment in which they were housed (natural or unnatural, except 3 fish housed in standard aquarium lighting).

	Host	N fish	SL (mm)		weight (g)		CF		age (days)		water depth (m)	
			mean	(min-max)	mean	(min-max)	mean	(min-max)	mean	(min-max)	mean	(min-max)
lab	*Pundamilia* sp. 'pundamilia-like'	30	96.91	(69.04-120.4)	27.62	(11.00-49.20)	2.76	(2.27-3.74)	1129	(286-2740)		
	natural light	14	96.47	(78.81-112.00)	26.06	(12.70-35.40)	2.67	(2.26-3.15)	1190	(286-2740)		
	unnatural light	15	97.62	(69.04-120.40)	28.98	(11.00-49.20)	2.85	(2.28-3.73)	1076	(286-1740)		
	Pundamilia sp. 'nyererei-like'	31	82.15	(40.67-101.3)	18.34	(1.80-36.20)	3.01	(2.22-5.07)	1115	(262-1931)		
	natural light	11	81.89	(72.08-91.30)	16.76	(9.40-23.30)	3.01	(2.22-5.07)	1132	(456-1928)		
	unnatural light	18	81.93	(40.67-101.30)	18.87	(1.80-36.20)	3.00	(2.52-3.86)	1057	(262-1931)		
	Pundamilia sp. 'hybrid'	25	87.96	(62.6-113.25)	21.81	(9.60-44.70)	3.21	(2.39-4.14)	1232	(342-2282)		
wild	*Pundamilia* sp. 'pundamilia-like'	39	88.3	(55.72-112)	19.02	(3.86-44.3)	2.75	(2.21-3.47)	na	na	1.21	(0.75-4.40)
	Pundamilia sp. 'nyererei-like'	37	69.28	(46.38-86)	8.04	(2.46-12.23)	2.55	(2.11-3.21)	na	na	3.35	(0.75-7.15)

Table S4.2
Sample size of fish hosts for each cross, separated by family and light treatment. The broad spectrum light treatment (mimicking shallow waters) resembles the natural visual environment of *Pundamilia* sp. 'pundamilia-like', the red-shifted light treatment (mimicking deeper waters) resembles that of *P.* sp. 'nyererei-like'. Six fish were housed in standard aquarium lighting (no tr.) and these were excluded from the light effect analysis. Family names are expressed as mother x father. Superscripted numbers indicate families with the same mothers; superscripted letters indicate families with the same fathers.

***P.* sp. 'pundamilia-like'**				***P.* sp. 'hybrid'**				***P.* sp. 'nyererei-like'**			
family	no tr.	deep	shallow	family	no tr.	deep	shallow	family	no tr.	deep	shallow
PP3 [j]			1	PN1 [i]		2	1	NN1	1	2	
PP4 [f]	1	1	1	PN2 [i]		1		NN3 [k]		1	3
PP7 [j]		3	2	PN8 [2k]	2		1	NN5 [8]	1	1	1
PP9 [4]		2	2	PN9			1	NN7 [i]			1
PP11 [1c]			1	PN10 [2]		1		NN18		1	3
PP12 [b]		1	1	PN11 [4g]		1	1	NN20 [3d]			3
PP13 [5b]		3	2	PN12 [4g]		2	4	NN21 [7d]		1	3
PP14 [4j]			2	PN13 [4g]	1		1	NN23 [e]		1	
PP15 [1g]		2		NP3 [8f]		1	1	NN24 [6e]			1
PP16 [5j]		2		NP6			2	NN26 [6h]		2	
PP17 [4j]		1	1	NP8 [7c]			2	NN28 [7h]		2	1
PP18 [4j]			1					NN29 [3a]			1
								NN30 [3a]			1
total	1	15	14	total	3	8	14	total	2	11	18

Table S4.3
Variance in infection between and within host species, in the wild and in the laboratory. Infection differences correspond to patterns of variance: when species differences in infection in the wild are statistically significant (shown in bold), variance between species is higher than variance within species.

		Wild			Lab		
		total	within	between	total	within	between
Prevalence	*Lamproglena monodi*	**1.533**	**0.237**	**1.296**	0.753	0.238	0.515
	Ergasilus. lamellifer	**0.967**	**0.206**	**0.761**	0.118	0.105	0.013
	Glochidia	0.264	0.245	0.019	0.096	0.077	0.019
Abundance	*Lamproglena monodi*	**13.794**	**2.701**	**11.093**	55.505	39.121	16.381
	Ergasilus lamellifer	**1.042**	**0.270**	**0.772**	0.480	0.239	0.241
	Glochidia	415.725	297.297	118.428	79.987	10.476	69.511

Table S4.4
Variation in infection prevalence and abundance between laboratory-bred *Pundamilia* sp. 'pundamilia-like' and *P.* sp. 'nyererei-like' raised in deep or shallow light treatments (*light*). The Minimum Adequate Model (MAM) was established by stepwise removal of non-significant variables (not shown). A model including the light treatment parameter was then tested against the MAM. *SL* fish standard length, *circ death* circumstances of death.

	prevalence					abundance				
	fixed effect	**Chisq**	**df**	**p**		**fixed effect**	**Chisq**	**df**	**p**	
Lamproglena monodi	SL	16.17	1	<0.001	***	age	12.29	1	<0.001	***
	circdeath	8.77	1	0.003	**	circdeath	13.41	1	<0.001	***
	light	0.00	1	0.978		light	0.16	1	0.686	
Ergasilus lamellifer	circdeath:age	6.30	2	0.043	*	1				
	light	0.07	1	0.797		light	0.71	1	0.399	
Glochidia	1					1				
	light	0.15	1	0.703		light	0.64	1	0.422	

Table S4.5
Differences in infection between laboratory-bred *Pundamilia* sp. 'pundamilia-like' (P. pun) and *P.* sp. 'nyererei-like' (P. nye) raised in natural or unnatural light conditions (*lightmatch*). **(a)** Differences in ectoparasite community composition, based on zero-adjusted Bray-Curtis distances (ANOSIM, 9999 permutations). Upper diagonal reports p-values (Benjamini-Hochberg corrected), lower diagonal R-values. **(b)** Variation in prevalence and abundance of individual ectoparasite taxa. The Minimum Adequate Model (MAM) was established by stepwise removal of non-significant variables (not shown). The effect of light condition (*lightmatch*) was also assessed separately against the MAM (shown in grey). **(c)** post hoc comparison (least square means) between the host species and light-matching conditions. *SL* fish standard length, *circ death* circumstances of death.

(a)	P. pun natural	P. pun unnatural	P. nye natural	P. nye unnatural
P. pun natural		0.677	0.710	0.677
P. pun unnatural	0.020		0.752	0.752
P. nye natural	-0.004	-0.043		0.752
P. nye unnatural	0.032	-0.028	-0.041	

Table S4.5 (continued)

	prevalence					abundance				
(b)	**fixed effect**	**Chisq**	**df**	**p**		**fixed effect**	**Chisq**	**df**	**p**	
Lamproglena monodi	SL	11.43	1	0.001	***	age	13.83	1	<0.001	***
	circdeath	9.61	1	0.002	**	circdeath	14.30	1	<0.001	***
	SL:circdeath	8.98	1	0.003	**					
	MAM + lightmatch	0.00	1	0.967		MAM + lightmatch	1.25	1	0.263	
Ergasilus lamellifer	1					1				
	MAM + lightmatch	0.62	1	0.429		MAM + lightmatch	0.13	1	0.717	
Glochidia	lightmatch	4.31	1	0.038	*	1				
						MAM + lightmatch	1.41	1	0.235	

		prevalence			abundance		
(c)	**comparison**	**estimate**	**t**	**p**	**estimate**	**t**	**p**
Lamproglena monodi	P. pun: nat vs. unnat	-0.15	-1.03	0.312	1.95	0.84	0.405
	P. nye: nat vs. unnat	0.15	0.88	0.385	1.36	0.58	0.566
	nat: P. nye vs. P. pun	0.08	0.41	0.682	-1.45	-0.59	0.556
	unnat: P. nye vs. P. pun	-0.22	-1.15	0.257	-0.86	-0.40	0.692
Ergasilus lamellifer	P. pun: nat vs. unnat	0.08	0.60	0.550	0.08	0.40	0.693
	P. nye: nat vs. unnat	0.71	0.54	0.594	0.05	0.25	0.802
	nat: P. nye vs. P. pun	0.04	0.28	0.779	0.13	0.62	0.537
	unnat: P. nye vs. P. pun	0.04	0.37	0.718	0.16	0.84	0.407
Glochidia	P. pun: nat vs. unnat	-0.20	-1.90	0.063	-8.80	-1.32	0.194
	P. nye: nat vs. unnat	-0.11	-1.02	0.315	-2.78	-0.40	0.692
	nat: P. nye vs. P. pun	0.00	0.00	1.000	0.00	0.00	1.000
	unnat: P. nye vs. P. pun	-0.09	-0.88	0.387	-6.02	-0.94	0.357

Table S4.6
Variation in the proportion of copepods carrying egg clutches among: **(a)** all non-hybrid host individuals (wild-caught in 2014 and laboratory-bred), **(b)** laboratory-bred hosts and interspecific hybrids (lab). The Minimum Adequate Model (MAM, in bold) was established by stepwise removal of non-significant variables (shown in previous rows). *SL* fish standard length, *wildlab* wild-caught or laboratory-bred fish.

			proportion of parasites with egg-clutches				
			fixed factor	**Chisq**	**df**	**p**	
(a)	*Lamproglena monodi*		species	0.54	1	0.464	
			wildlab	0.45	1	0.505	
			species:wildlab	4.83	3	0.184	
			abundance	0.08	1	0.771	
		MAM	**SL**	**6.62**	**1**	**0.010**	*
	Ergasilus lamellifer		species	0.57	1	0.449	
			wildlab	21.41	1	<0.0001	***
			species:wildlab	19.04	3	<0.001	***
			SL	0.09	1	0.765	
			abundance	0.60	1	0.439	
		MAM	**wildlab**	**34.61**	**1**	**<0.0001**	***
			SL	**7.11**	**1**	**0.008**	**
(b)	*Lamproglena monodi*		species	0.14	2	0.931	
			SL	0.57	1	0.565	
			abundance	0.08	1	0.771	
		MAM	**1**				
	Ergasilus lamellifer		species	0.03	2	0.985	
			SL	0.00	1	0.984	
			abundance	0.05	1	0.816	
		MAM	**1**				


5

5

Microhabitat distribution and species relationships of ectoparasites on the gills of cichlid fish in Lake Victoria, Tanzania

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* contributed equally

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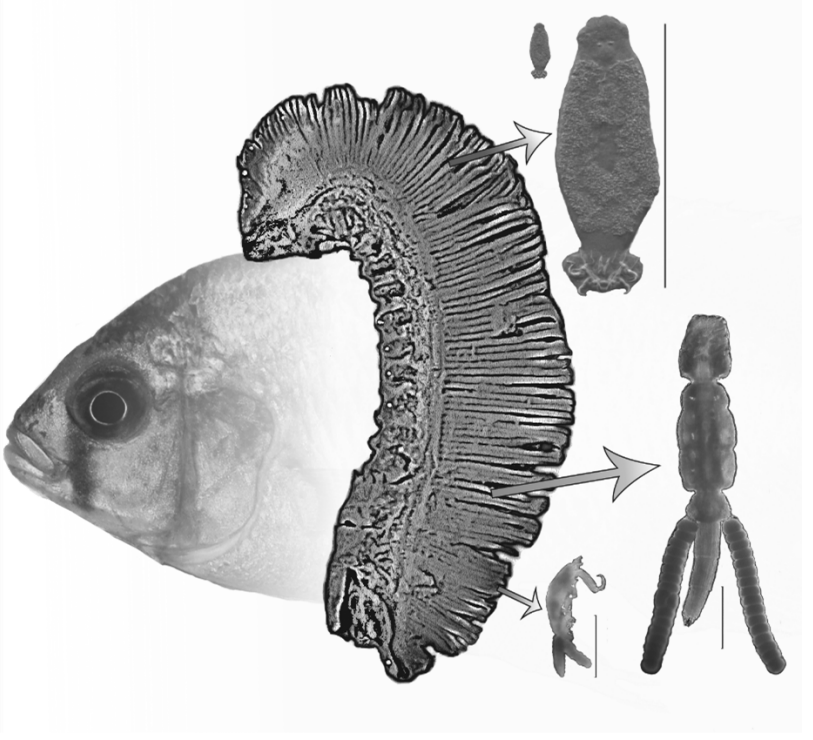


ABSTRACT

Heterogeneous exposure to parasites may contribute to host species differentiation. Hosts often harbour multiple parasite species which may interact and thus modify each other's effects on host fitness. Antagonistic or synergistic interactions between parasites may be detectable as niche segregation within hosts. Consequently, the within-host distribution of different parasite taxa may constitute an important axis of infection variation among host populations and species. We investigated the microhabitat distributions and species interactions of gill parasites (four genera) infecting 14 sympatric cichlid species in Lake Victoria, Tanzania. We found that the two most abundant ectoparasite genera (the monogenean *Cichlidogyrus* spp. and the copepod *Lamproglena monodi*) were non-randomly distributed across the host gills and their spatial distribution differed between host species. This may indicate microhabitat selection by the parasites and cryptic differences in the host-parasite interaction among host species. Relationships among ectoparasite genera were synergistic: the abundances of *Cichlidogyrus* spp. and the copepods *L. monodi* and *Ergasilus lamellifer* tended to be positively correlated. In contrast, relationships among species of *Cichlidogyrus* were antagonistic: the abundances of species were negatively correlated. Together with niche overlap, this suggests competition among species of *Cichlidogyrus*. We also assessed the reproductive activity of the copepod species (the proportion of individuals carrying egg clutches), as it may be affected by the presence of other parasites and provide another indicator of the species specificity of the host-parasite relationship. Copepod reproductive activity did not differ between host species and was not associated with the presence or abundance of other parasites, suggesting that these are generalist parasites, thriving in all cichlid species examined from Lake Victoria.

Keywords:

host-parasite interaction, parasite-parasite interaction, niche selection, Monogenea, Copepoda, Cichlidae

5.1. INTRODUCTION

Parasites can be important agents of selection on host populations, affecting host fitness through effects on e.g. host growth, reproduction and survival (Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018). They engage with their hosts in coevolutionary arms races of adaptation and counter-adaptation (Decaestecker et al., 2007). Host species occupying different ecological niches are exposed to different parasites, potentially resulting in different infection profiles (here defined as the combination of parasite species diversity and abundance in a given host population (Knudsen et al., 2004; Pegg et al., 2015; Hablützel et al., 2017; Hayward et al., 2017). Differences in exposure may lead to genetic divergence in immunity among host populations and species, possibly contributing to host reproductive isolation (Hamilton & Zuk, 1982; Landry et al., 2001; Nosil et al., 2005; Maan et al., 2008; Eizaguirre et al., 2011; Karvonen & Seehausen, 2012).

Several studies have reported differences in infection (in terms of parasite species identity and numbers) between closely related host species (Morand et al., 2015). If parasites impose a fitness cost, such differences may contribute to host divergence in resistance or tolerance, promoting reproductive isolation and perhaps speciation (Karvonen & Seehausen, 2012). Most studies of parasite-mediated divergent selection are based on parasite counts: differences between host populations in the prevalence, abundance, and intensity of various parasite taxa (e.g. Forbes et al., 1999; Medel, 2000; Maan et al., 2008; Konijnendijk et al., 2013). This approach presents two limitations. First, the parasite count approach ignores possible differences between host species in the spatial distribution of parasites. Some parasitic groups, for example monogeneans, are not only specialised to host species, but also to specific microhabitats within the host (Šimková & Morand, 2015). This may be driven by spatial variation in competition intensity, attachment opportunities, resource quality or access to mates (Rohde, 1994), or host spatial variation in defence mechanisms. We hypothesize that host species that are infected by the same parasite species in similar numbers may actually differ in how these parasites are spatially distributed. We suppose that this variation could result from the specific host morphology, without involving specific adaptations by the parasite. Alternatively, we may expect that differences in host characteristics (morphology, behaviour, physiology) could give rise to adaptation of the parasites, generating host species-specific parasite 'ecotypes', occupying different niches in different hosts. Such patterns can be detected only by investigating the within-host spatial distribution of parasites. Here, we expand on our previous studies of parasite-mediated divergence in African cichlid fish (**Maan et al., 2008; Karvonen et al., 2018; Gobbin et al., 2020b**; **Gobbin et al., in prep**.), by exploring parasite microhabitat segregation in a species assemblage of cichlids from Lake Victoria, Tanzania.

Second, parasite count measures are based on the assumption that parasites are independent of each other. However, hosts very frequently carry several parasite species at the same time (López-Villavicencio et al., 2007; Poulin, 2007; Taerum et al., 2010; Griffiths et al., 2011; Schmid-Hempel, 2013). These parasites may interact, with consequences for both host-parasite and parasite-parasite dynamics Poulin, 2001; Mideo, 2009; Alizon et al., 2013). In the presence of competitors, parasite infection sites may change, thereby reducing interference (Holmes, 1973; Poulin, 2001). If parasite-parasite competition is strong and consistent over evolutionary time, then such niche segregation may become genetically fixed, resulting in a permanent change in the fundamental ecological niche (Holmes, 1973; for ecological character displacement see Brown & Wilson, 1956; Schluter, 2000a). Competition-driven niche segregation has been observed in gastrointestinal helminths of fish (Vidal-Martínez & Kennedy, 2000; Karvonen et al., 2006) and birds (Bush & Holmes, 1986), in arthropod ectoparasites of birds (Choe & Kim, 1988, 1989) and in oxyurid nematodes infecting cockroaches (Adamson & Noble, 1992). In other host-parasite systems, this phenomenon was not observed, such as in 23 metazoan species of marine fish (Mouillot et al., 2003) and nine monogenean species in roach (Šimková et al., 2000).

Positive (synergistic) and negative (antagonistic) interactions among parasites modify each other's effects on host individuals (Graham, 2008; Thumbi et al., 2013), with possible consequences at host population level (Rohani et al., 2003; Graham, 2008; Telfer et al., 2008; Mideo, 2009). For example, simultaneous and subsequent co-infections may facilitate parasite infection through mechanical damage (Bandilla et al., 2006) or through immunosuppression of the host (immunity-mediated facilitation, Jokela et al., 2000; Graham, 2008; Ezenwa et al., 2010; Karvonen et al., 2012). Such positive interactions are relatively common (Lotz & Font, 1991; Šimková et al., 2000; Dallas et al., 2019). Negative interactions can occur, especially between parasites co-infecting the same host tissue, competing for resources and space (resource-mediated competition; Lello et al., 2004; Graham, 2008; Daniels et al., 2013; Vaumourin et al., 2015; Dallas et al., 2019). Negative interactions can also arise from cross-immunity: one parasite elicits an immune response that is also effective against other species of parasites (immunity-mediated competition; Lello et al., 2004; Porrozzi et al., 2004). Although uncommon, interference competition can also take place: compounds secreted by a parasite can negatively affect the fitness of a competitor (Behnke et al., 2001; Cox, 2001).

Cichlid fish of the Great East African Lakes (Lakes Malawi, Tanganyika and Victoria) form a well-studied example of adaptive radiation (Kornfield & Smith, 2000; Kocher, 2004; Seehausen, 2006), with a high diversity in macrohabitat, microhabitat and trophic specialization (Sturmbauer & Meyer, 1992; Bouton et al., 1997; Genner et al., 1999). Previous studies have shown that cichlids are typically infected by multiple species of parasites, with different parasite communities and abundances between species (Lake Victoria: Maan et al., 2008; Karvonen et al., 2018; **Gobbin et al., 2020b**; Lake Tanganyika: Vanhove et al., 2015; Hablützel et al., 2017; Lake Malawi: Blais et al., 2007). Consequently, it has been suggested that cichlid parasites may contribute to host

diversification (reviewed in Vanhove et al., 2016; **Gobbin et al., 2020b**). However, large scale investigations of parasite ecology and interspecific interactions between parasite taxa are scarce. Previous studies of microhabitat distribution of gill parasites in cichlids and other fish suggest that parasites with low within-host abundances are not saturating the available niche space in the gills, and thus they lack competition (Rohde, 1991; Rohde, 1994). Consequently, the observed spatial niche restriction could be driven by other processes than competition, such as facilitation of mate finding (in siganid fishes, Geets et al., 1997; in pomacentrid fishes, Lo, 1999). Although monogeneans were long assumed to lack interspecific competition (e.g. Morand et al., 2002; Rohde, 2002), some studies found evidence for competition-driven microhabitat selection and reduced niche overlap between monogenean species (*Dactylogyrus carpathicus* and *Dactylogyrus malleus*; Kadlec et al., 2003 and *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini*; Matějusová et al., 2003).

In the present study, we aimed to determine if there is cryptic differentiation and microhabitat specialisation of ectoparasites infecting 14 sympatric Lake Victoria cichlid species. We investigated infection of *Lamproglena monodi* Capart, 1944 (Copepoda: Cyclopoida: Lernaeidae)*, Ergasilus lamellifer* Fryer, 1961 (Copepoda: Poecilostomatoida: Ergasilidae)*,* and *Cichlidogyrus* Paperna, 1960 (Monogenea: Dactylogyridea: Dactylogyridae) (the latter at both genus and species level). Several species of *Cichlidogyrus* (Monogenea, Dactylogyridae) occur in Lake Victoria, most of which are not formally described. This flatworm gill parasite primarily infests members of the family Cichlidae (Paperna, 1960) (but also killifishes within *Aphyosemion* (Messu Mandeng et al., 2015) and the nandid *Polycentropsis abbreviata* (Pariselle & Euzet, 2009)). Some species of *Cichlidogyrus* are specific to a single cichlid species or a few closely related species (Pariselle & Euzet, 2009; Roux & Avenant-Oldewage, 2010; Mendlová & Šimková, 2014). Others have a broad host range (Jorissen et al., 2018b). The presence of several cryptic species of *Cichlidogyrus* was previously revealed by molecular investigations in cichlids from the Ivory Coast (Pouyaud et al., 2006). Many species descriptions of *Cichlidogyrus* only report host species, and the gills in general as the infection site, and no other ecological data; here we also report within-host microhabitat distribution within the gills.

We explored the relationships between different parasite taxa and how they differ between host species. If parasite taxa are competing, their abundances may be negatively correlated. A positive correlation would emerge if parasite interactions are synergistic. Differences between host species in the strength and/or direction of such parasite associations could indicate that the host-parasite relationship is species-specific.

Finally, we also investigated whether the reproductive activity of copepods differs between host species and whether this may be influenced by the presence of conspecific or heterospecific parasites.

5.2. METHODS

5.2.1. Fish collection

Cichlid fish were collected in June-October 2014 at Makobe Island, in southern Lake Victoria, Tanzania, by angling and with gillnets of variable mesh sizes, set at different depths (0.5-19.0 m). We collected 332 fishes from 14 sympatric cichlid species belonging to the Lake Victoria haplochromine radiation, with different ecological specializations (i.e. diet and water depth distribution, Witte & van Oijen, 1990; Seehausen, 1996b; Bouton et al., 1997; Seehausen & Bouton, 1998; **Table S5.1**) and different levels of genetic differentiation among them (Wagner et al., 2012a; Karvonen et al., 2018). Since females are difficult to identify in the field, only males were considered. Fish were euthanised with an overdose of 2-phenoxyethanol (2.5 ml/l) immediately after capture. In the field, immediately after collection, 148 fish (whole body) were preserved in 4% formalin and subsequently transferred to increasing concentrations of ethanol (final concentration 70%), 184 fish were directly preserved in 100% ethanol (for future genetic analysis). Samples were shipped to Europe for analyses. Each individual fish was measured (standard length (SL), body depth (BD), to the nearest 0.1 mm) and weighed (to the nearest 0.1 g) on the same day as parasite screening (901 ± 129 days after collection (mean ± S.D.)). We calculated individual fish condition factor (CF) as CF = 100*(weight/SL^3) (Sutton et al., 2000). Sampling was conducted with permission from the Tanzania Commission for Science and Technology (COSTECH - No. 2013-253-NA-2014-117).

5.2.2. Parasite screening

We examined the gills on the right side of each fish, under a dissecting stereoscope. All macroparasites were counted and identified (following Paperna, 1996 and monogenean literature: Vanhove et al., 2011; Muterezi Bukinga et al., 2012). We observed 1414 individuals in five ectoparasite taxa: *Cichlidogyrus* spp. Paperna, 1960 (Monogenea: Dactylogyridea: Dactylogyridae), *Gyrodactylus sturmbaueri* Vanhove, Snoeks, Volckaert & Huyse, 2011 (Monogenea: Gyrodactylidea: Gyrodactylidae), *Lamproglena monodi* Capart, 1944 (Copepoda: Cyclopoida: Lernaeidae), *Ergasilus lamellifer* Fryer, 1961 (Copepoda: Poecilostomatoida: Ergasilidae), glochidia mussel larvae (Bivalvia: Unionoidea). *Gyrodactylus sturmbaueri* was found only once and therefore not included in analyses. The attachment site on the gills was recorded for *Cichlidogyrus* spp., *L. monodi* and *E. lamellifer* (but not for glochidia; **Table S5.2**), according to a subdivision of each gill arch into nine microhabitats (resulting in a total of 36 gill microhabitats; Gelnar et al., 1990). This subdivision was based on coarser spatial units: gill arches (from anterior to posterior: I, II, III, IV), longitudinal segments (dorsal, medial, ventral) and vertical areas (proximal, central, distal; from the tip of the gill filaments to the gill bar) (**Fig. 5.1a**). The presence or absence of egg clutches in copepod females was recorded.

5.2.3. Species identification of *Cichlidogyrus*

For morphological identification of *Cichlidogyrus* we randomly selected a subset of specimens (*n*=213) from 11 host species that each carried more than 10 parasite individuals. We aimed to identify 15 specimens of *Cichlidogyrus* per host species, by sampling all worms infesting each fish individual (1<*n*>7) from a randomly selected pool of each host species. If the total number of worms available per host population was less than 15, then all worms of that host population were identified (see **Table S5.1** for sample sizes).

Specimens of *Cichlidogyrus* were mounted on slides in Hoyer's medium, after prior treatment with 20% sodium dodecyl sulphate to soften tissues. They were examined with a microscope (Olympus BX41TF) under 1000x magnification using differential interference phase contrast. Although most of the species of *Cichlidogyrus* that we found are not formally described, species can be discriminated based on the shape and size of sclerotized parts of the attachment organ (haptor) and, in particular, on those of the male copulatory organ (MCO) (e.g. Grégoir et al., 2015; **Gobbin et al., 2020b**). Morphological assessment of worms belonging to *Cichlidogyrus* revealed the presence of five different species *Cichlidogyrus bifurcatus*, *C. nyanza*, *C. furu*, *C. pseudodossoui* and *C. vetusmolendarius* (described in **chapter 6**).

5.2.4. Data analysis

Parasite spatial distribution

To investigate the spatial distribution of each parasite taxon and of each species of *Cichlidogyrus* on the 36 gill microhabitats, we used generalized linear models in R (R Core Team, 2019). Fixed effects included gill microhabitat and the total abundance of the respective parasite per fish individual, to correct for interindividual variation in infection. Since the preservation method (formalin or ethanol) had an effect on the intensity of one of the parasite taxa (*Cichlidogyrus* spp., **Table S5.3**), we included that as a fixed effect. Random effects included: fish individual identity, to account for repeated sampling (as each fish individual could be infected by several parasites) and host species, to control for pseudoreplication. A random effect at the level of observation was included to correct for overdispersion. We determined the significance of fixed effects by likelihood ratio tests (LRTs). Host species represented by fewer than five individuals were excluded from analyses (14 host species analysed at the parasite higher taxon level, seven at the *Cichlidogyrus* species level).

To obtain a general overview of the parasite spatial distributions and assess host species differences in parasite spatial distribution, we also analysed coarser spatial units than the 36 microhabitats considered above. These are: gill arches (I, II, III, IV), longitudinal segments (dorsal, medial, ventral) and vertical areas (proximal, central, distal) (**Fig. 5.1a**). We used generalized

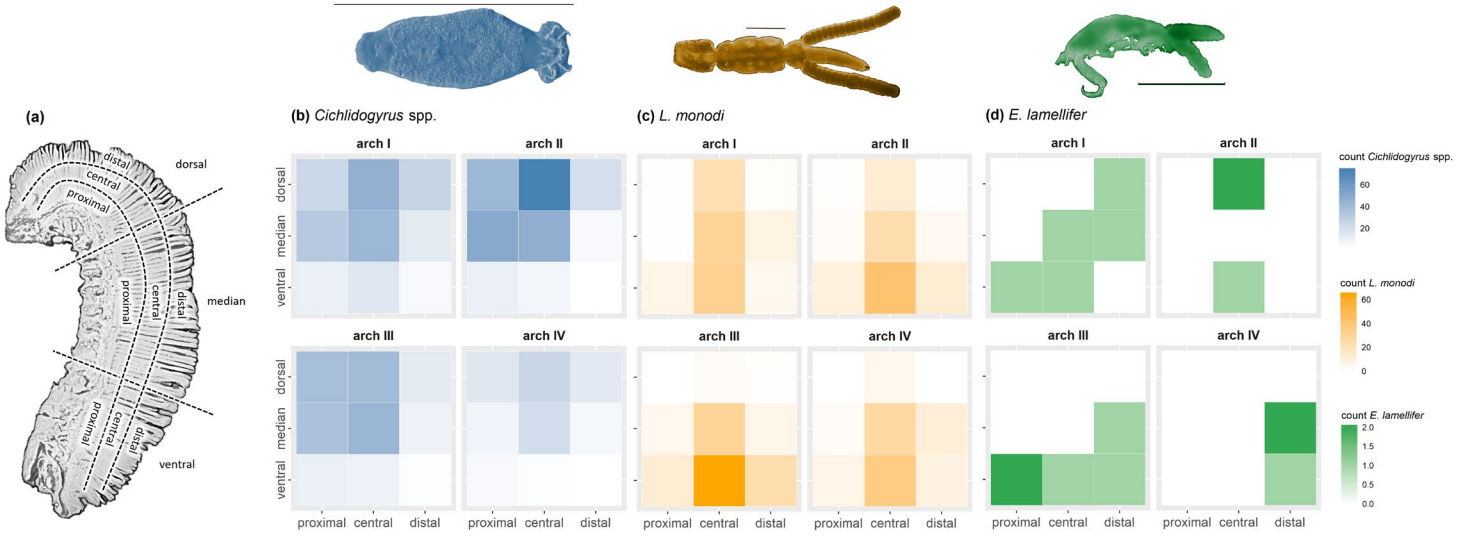


Figure 5.1
Gill microhabitat distributions of three ectoparasite taxa infecting cichlids sampled at Makobe Island (Lake Victoria, Tanzania). **(a)** Spatial subdivision of gill arches into longitudinal segments (dorsal, medial, ventral) and vertical areas (proximal, central, distal). Microhabitat distribution, expressed as abundance, of **(b)** *Cichlidogyrus* spp., **(c)** *Lamproglena monodi* and **(d)** *Ergasilus lamellifer*. Microscope photographs of the studied gill parasites (dorsal view for *Cichlidogyrus* and *L. monodi*, lateral view for *E. lamellifer*; scale bars are 500 µm).

linear models, followed by post-hoc Tukey tests. Fixed effects included host species (to account for species differences in parasite abundance), gill microhabitat (four arches or three longitudinal segments or three vertical areas) and their interactions, as well as the total abundance of the respective parasite per fish individual (to correct for interindividual variation in infection). Since the preservation method (formalin or ethanol) had an effect on the intensity of one of the parasite taxa (*Cichlidogyrus* spp., **Table S5.3**), we included that as a fixed effect. In particular, the interaction species:microhabitat indicates whether the spatial distribution differs between host species. This was not assessed for the 36 sites analysis as comparisons were too numerous to achieve sufficient statistical power. Random effects included fish individual identity, to account for repeated sampling (as each fish individual could be infected by several parasites). A random effect at the level of observation was also included to correct for overdispersion. We determined the significance of fixed effects by LRTs.

To investigate if the overall spatial distribution pattern was present in each host species or only in some, we applied the same models separately on each host species. The significance level was corrected for pseudo-replication (Benjamini & Hochberg, 1995).

Interactions between parasites

We used generalized linear models to investigate if the abundance of a given parasite genus or a species of *Cichlidogyrus* was correlated with the abundance of another genus or species. Fixed effects included host species and the abundance of each parasite genus. In parasite genus models (not *Cichlidogyrus* species models due to low sample size) we also included as fixed effects all interaction terms between host species and abundance of each parasite genus. We selected the Minimum Adequate Model (MAM) by stepwise removal of non-significant variables, determined by LRT. Where overdispersion was detected, we corrected the standard errors using a quasipoisson model (Zuur et al., 2009). Host species represented by fewer than 10 fish individuals were excluded from analysis at parasite higher taxon level. This was not done for the analysis of species of *Cichlidogyrus,* to allow comparisons between a sufficient number of different host species.

To investigate if interspecific interactions among parasite genera (not species of *Cichlidogyrus* due to low sample size) were present in each host species or only in some, we applied the same models separately on each host species. Significance level was corrected for pseudo-replication (Benjamini & Hochberg, 1995).

Reproductive activity of copepods

Female parasitic copepods attached to gills produce egg clutches appended to their body. We used the presence of egg clutches as a proxy for copepod reproductive activity. This may provide indications of species specificity of the host-parasite relationship (Paperna, 1996). We compared the proportion of copepods carrying egg clutches between host species using generalized linear models. Fixed effects included host species, host SL and host CF, capture water depth, abundance of conspecifics and of heterospecifics, fish preservation method (formalin versus ethanol) and days elapsed between fish collection and parasite screening. As above, we determined the significance of fixed effects by LRT and we used Tukey's post-hoc test to obtain parameter estimates.

5.3. RESULTS

5.3.1. Non-random spatial distribution on fish gills: parasite genera

The spatial distribution of *Cichlidogyrus* spp. and of *L. monodi* was non-random across the 36 gill attachment sites (**Table 5.1**). In contrast, the spatial distribution of *E. lamellifer* did not significantly deviate from random, probably due to the low sample size (18 parasites in 248 fish individuals).

When considering the lower resolution distributions over gill arches, segments and areas, we also observed a non-random spatial distribution of *Cichlidogyrus* spp. and *L. monodi* (**Table 5.1**). Overall, *Cichlidogyrus* spp. were less abundant on the fourth gill arch, compared with the three other arches, whereas *L. monodi* were more abundant on the third arch than on the fourth. Distribution patterns of longitudinal segments were reversed for *Cichlidogyrus* spp. and *L. monodi*: the former were more abundant on the dorsal segment and less on the ventral one, while the latter were more abundant on the ventral segment and less on the dorsal one (**Table 5.1**, **Fig. 5.2**). Both *Cichlidogyrus* spp. and *L. monodi* were more abundant in the central area, but this was more pronounced in the latter. *Ergasilus lamellifer* followed the longitudinal distribution pattern of the other copepod, *L. monodi*, with an increasing abundance towards more ventral segments.

The non-random distributions of *Cichlidogyrus* spp. and *L. monodi* were also observed when testing each host species separately (**Table S5.4**). *Cichlidogyrus* spp. were non-randomly distributed across all gill microhabitats in eight out of 13 infected host species (**Fig. 5.3**); *L. monodi* were non-randomly distributed across all gill microhabitats in 12 out of 14 infected host species (**Fig. 5.3**). For the lower resolution distributions: *Cichlidogyrus* spp. were non-randomly distributed across vertical areas in nine out of the 13 infected host species, *L. monodi*

Table 5.1

Differences in the spatial distribution of parasites on the gills of cichlids inhabiting Makobe Island (all 36 microhabitats, gill arches, longitudinal segments and vertical areas). The reported contribution of each fixed effect was assessed through ANOVA. For all microhabitat analyses, starting models included parasite location on the gill and total parasite intensity per host individual and preservation method (random effects: host species, fish individual identity, number of observations). For other analyses, starting models included host species, parasite location on the gill, their interaction term and total number of parasite individuals per host individual (N parasites) and preservation method (random effects: fish individual identity, number of observations). Tukey pairwise comparison between spatial locations (except all 36 microhabitats) revealed significant parasite microhabitat selection.

	Parasite	Fixed effect	Chi sq	df	p		Comparison	estimate	Z	p	
all micro.habitats (36)	*Cichlidogyrus* spp.	site36	215.29	35	<0.0001	***					
		nr parasites	216.98	1	<0.0001	***					
		preservation	0.11	1	0.745						
	Lamproglena monodi	site36	252.90	35	<0.0001	***					
		nr parasites	135.90	1	<0.0001	***					
		preservation	0.01	1	0.939						
	Ergasilus lamellifer	site36	1.80	35	1.000						
		nr parasites	NA								
		preservation	0.00	1	1.000						
gill arches (4)	*Cichlidogyrus* spp.	species	16.69	12	0.162		II vs. I	0.15	1.36	0.522	
		arch	46.61	3	<0.0001	***	III vs. I	-0.06	-0.49	0.962	
		nr parasites	239.10	1	<0.0001	***	IV < I	-0.75	-5.52	<0.001	***
		species:arch	61.31	36	0.005	**	III vs. II	-0.21	-1.85	0.248	
		preservation	0.00	1	0.977		IV < II	-0.90	-6.80	<0.001	***
							IV < III	-0.69	-5.08	<0.001	***
	Lamproglena monodi	species	26.88	13	0.013	*	II vs. I	0.01	0.07	0.999	
		arch	7.42	3	0.060	.	III > I	0.29	2.31	0.096	.
		nr parasites	303.24	1	<0.0001	***	IV vs. I	-0.09	-0.62	0.925	
		species:arch	41.24	39	0.373		III vs. II	0.28	2.24	0.111	
		preservation	0.22	1	0.640		IV vs. II	-0.10	-0.69	0.901	
							IV < III	-0.38	-2.92	0.018	*

Table 5.1 (continued)

	Parasite	Fixed effect	Chi sq	df	p		Comparison	estimate	Z	p	
gill arches (4)	*Ergasilus*	species	NA				II vs. I	-0.51	-0.70	0.897	
	lamellifer	arch	NA				III vs. I	0.00	0.00	1.000	
		nr parasites	NA				IV vs. I	-0.51	-0.70	0.897	
		species:arch	NA				III vs. II	0.51	0.70	0.897	
		preservation	NA				IV vs. II	0.00	0.00	1.000	
							IV vs. III	-0.51	-0.70	0.897	
longitudinal segments (3)	*Cichlidogyrus*	species	27.80	12	0.006	**	median < dorsal	-0.19	-2.25	0.062	.
	spp.	segment	115.51	2	<0.0001	***	ventral < dorsal	-1.43	-11.86	<0.001	***
		nr parasites	291.78	1	<0.0001	***	ventral < median	-1.24	-10.13	<0.001	***
		species:segment	47.81	24	0.003	**					
		preservation	0.03	1	0.870						
	Lamproglena	species	2.49	14	0.999		median > dorsal	1.13	6.85	<0.0001	***
	monodi	segment	103.86	3	<0.0001	***	ventral > dorsal	1.68	10.77	<0.0001	***
		nr parasites	203.40	1	<0.0001	***	ventral > median	0.55	5.39	<0.0001	***
		species:segment	35.54	26	0.100						
		preservation	0.00	1	0.994						
	Ergasilus	species	2.80	9	0.972		median vs. dorsal	0.51	0.70	0.762	
	lamellifer	segment	0.00	3	1.000		ventral vs. dorsal	0.98	1.45	0.313	
		nr parasites	NA	0	NA		ventral vs. median	0.47	0.82	0.686	
		species:segment	0.00	16	1.000						
		preservation	0.00	1	1.000						
vertical areas (3)	*Cichlidogyrus*	species	15.14	12	0.234		central > proximal	0.31	3.22	0.004	**
	spp.	area	79.69	2	<0.0001	***	distal < proximal	-0.80	-6.60	<0.001	***
		nr parasites	277.66	1	<0.0001	***	distal < central	-1.11	-9.44	<0.001	***
		species:area	95.16	24	<0.0001	***					
		preservation	0.05	1	0.823						

Table 5.1 (continued)

	Parasite	Fixed effect	Chi sq	df	p		Comparison	estimate	Z	p	
vertical areas (3)	*Lamproglena monodi*	species	57.16	34	0.008	**	central > proximal	1.86	12.34	<0.001	***
		area	204.08	23	<0.0001	***	distal > proximal	0.49	2.74	0.016	*
		nr parasites	202.53	1	<0.0001	***	distal < central	-1.37	-11.16	<0.001	***
		species:area	48.09	26	0.005	**					
		preservation	0.00	1	0.996						
	Ergasilus lamellifer	species	4.37	9	0.886		central vs. proximal	0.60	0.98	0.587	
		area	2.56	3	0.464		distal vs. proximal	0.85	1.23	0.434	
		nr parasites	NA	0			distal vs. central	0.15	0.28	0.958	
		species:area	0.00	16	1.000						
		preservation	0.00	1	1.000						

· P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001; df, degrees of freedom; NA, not available.

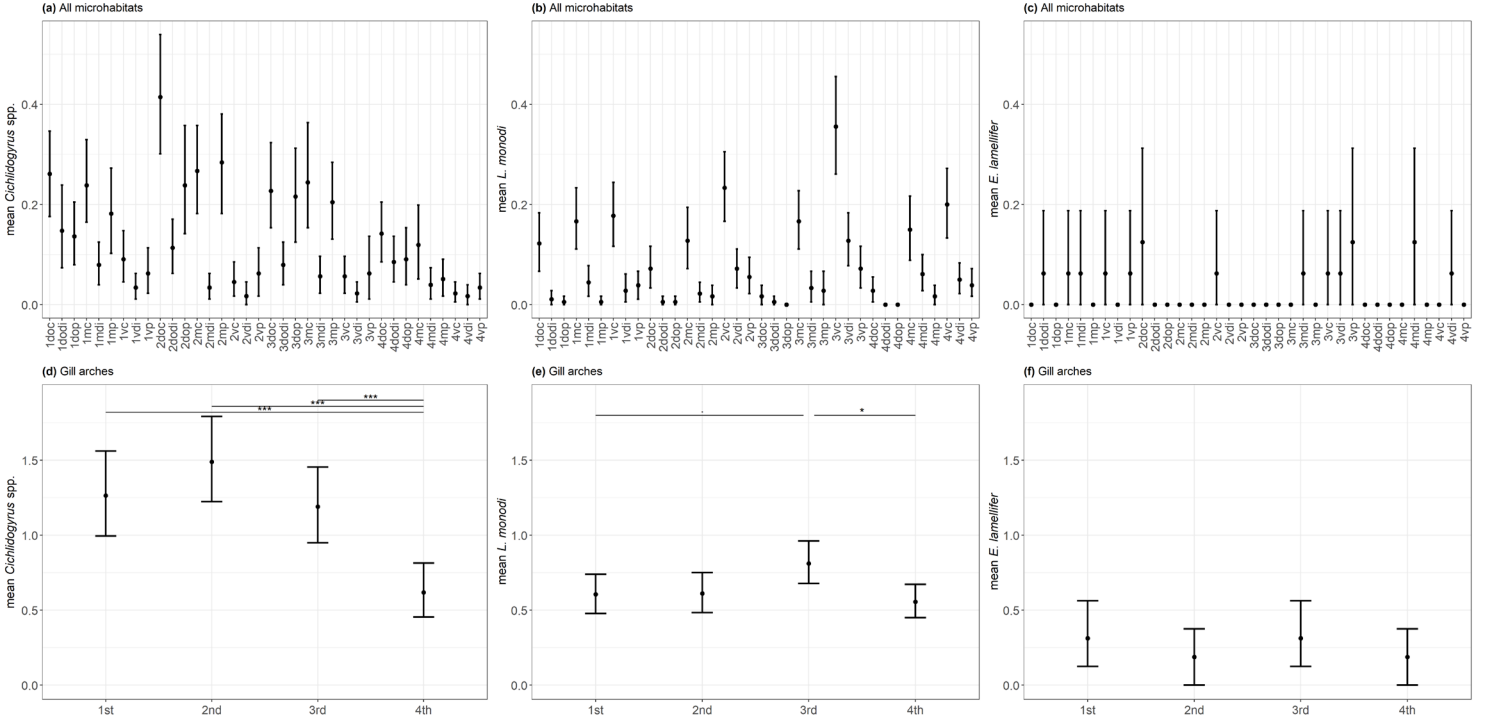
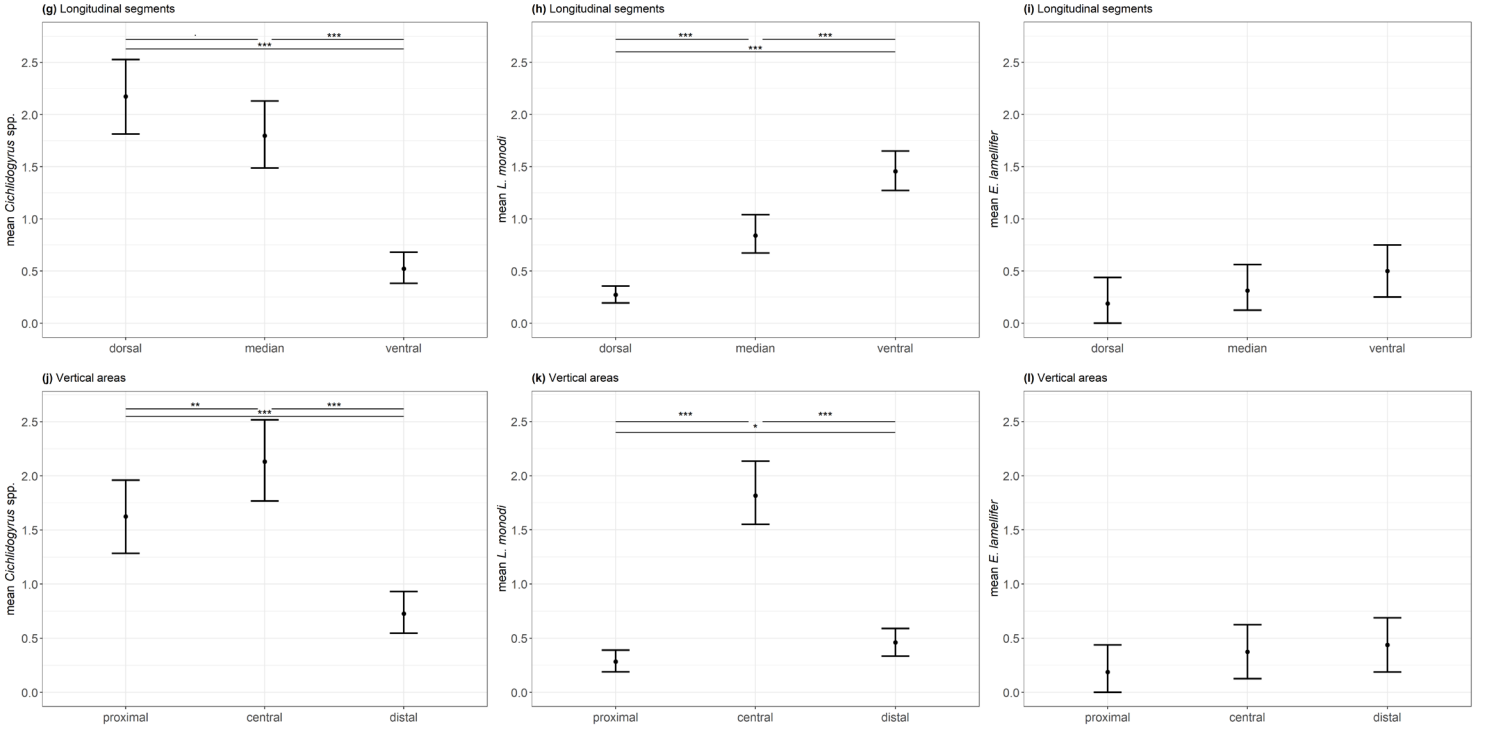


Figure 5.2
Spatial distribution of *Cichlidogyrus* spp. (left panels), *Lamproglena monodi* (middle) and *Ergasilus lamellifer* (right) infecting cichlid gills at Makobe Island. **(a-c)** all 36 microhabitats, **(d-f)** gill arches, **(g-i)** longitudinal segments and **(j-l)** vertical areas. Asterisks indicate a significant difference in parasite spatial distribution between microhabitats ($p<0.05$) (except in (a-c), where post-hoc tests were not performed).



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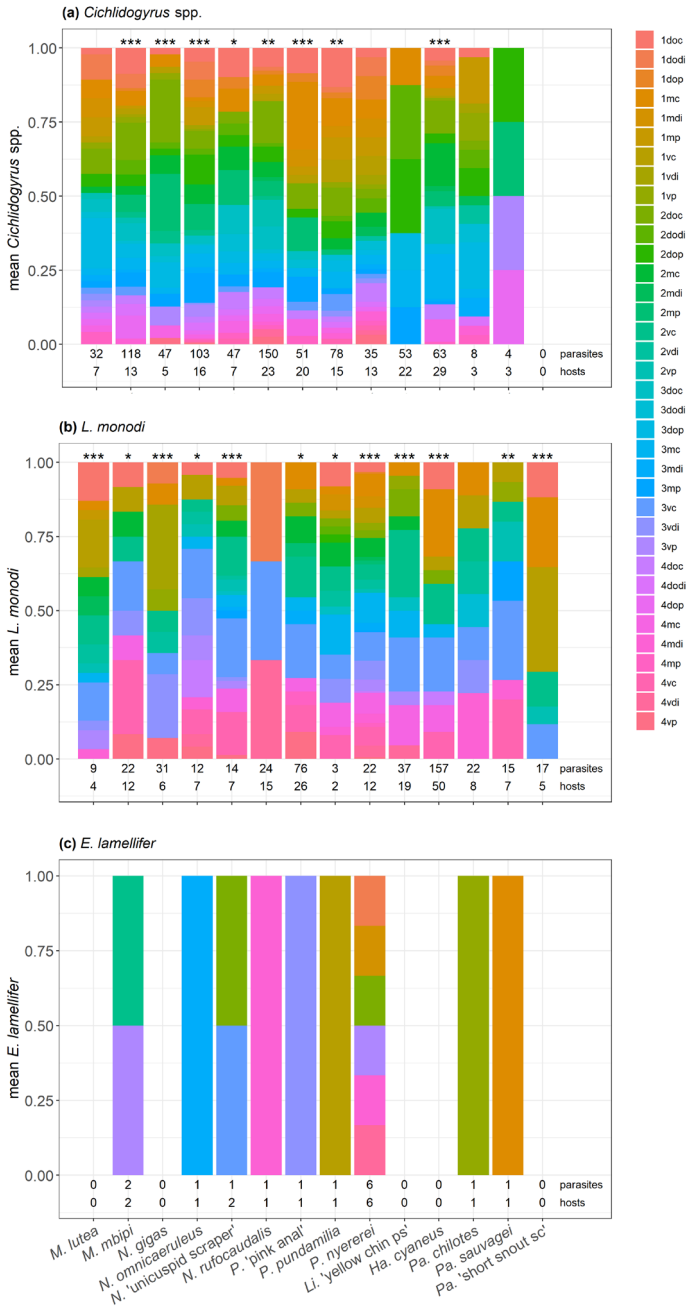


Figure 5.3
Within-host spatial distribution over 36 gill microhabitats of **(a)** *Cichlidogyrus* spp., **(b)** *Lamproglena monodi* and **(c)** *Ergasilus lamellifer*, in 14 cichlid host species inhabiting Makobe Island. Asterisks indicate a significant within-species non-random distribution ($p<0.05$). The total number of parasites and of infected host individuals per species are reported.

Table 5.2

Differences in spatial distribution on fish gills (all 36 microhabitats, gill arches, longitudinal segments and vertical area) of species of *Cichlidogyrus* infecting cichlids inhabiting Makobe Island. The reported contribution of each fixed effect was assessed through ANOVA. For all microhabitat analyses, starting models included parasite location on the gill and total parasite intensity per host individual (N parasites) (random effects: host species, fish individual identity, number of observations). For other analyses, starting models included host species, parasite location on the gill, their interaction term and total parasite intensity per host individual (N parasites) (random effects: fish individual identity). Tukey pairwise comparison between spatial locations (except all 36 microhabitats) revealed significant parasite microhabitat selection.

	Cichlidogyrus	**Fixed effect**	**Chi sq**	**df**	**P**		**Comparison**	**estimate**	**Z**	**p**	
all microhabitats (36)	*C. nyanza*	site36	85.07	35	<0.001	***					
		nr parasites	0.15	1	0.700						
	C. furu	site36	23.24	35	0.936						
		nr parasites	0.09	1	0.766						
gill arches (4)	*C. nyanza*	species	0.00	6	1.000		II vs. I	0.02	1.30	0.560	
		arch	20.55	3	<0.001	***	III vs. I	0.00	0.00	1.000	
		nr parasites	0.00	1	1.000		IV < I	-0.14	-3.00	0.014	*
		species:arch	36.46	18	0.006	**	III vs. II	-0.06	-1.30	0.560	
							IV < II	-0.21	-4.30	<0.001	***
							IV < III	-0.14	-3.00	0.014	*
	C. furu	species	0.00	6	1.000		II vs. I	-0.11	-1.44	0.472	
		arch	13.09	3	0.004	**	III < I	-0.14	-1.86	0.247	
		nr parasites	0.00	1	1.000		IV < I	-0.27	-3.51	0.003	**
		species:arch	29.40	18	0.044	*	III > II	-0.03	-0.41	0.976	
							IV < II	-0.16	-2.06	0.165	
							IV < III	-0.13	-1.65	0.268	

Table 5.2 (continued)

	Cichlidogyrus	Fixed effect	Chi sq	df	P		Comparison	estimate	Z	p	
longitudinal segments (3)	*C. nyanza*	species	0.36	6	0.999		median vs. dorsal	0.09	1.89	0.142	
		segment	63.68	2	<0.001	***	ventral < dorsal	-0.28	-5.67	<0.001	***
		nr parasites	0.22	1	0.639		ventral < median	-0.39	-7.91	<0.001	***
		species:segment	25.83	12	0.011	*					
	C. furu	species	0.31	6	0.999		median > dorsal	0.07	1.69	0.208	
		segment	1.75	2	0.417		ventral > dorsal	-0.27	-6.35	<0.0001	***
		nr parasites	0.06	1	0.806		ventral > median	-0.34	-8.04	<0.0001	***
		species:segment	18.68	12	0.096	.					
vertical areas (3)	*C. nyanza*	species	0.40	6	0.999		central > distal	0.54	11.54	<0.001	***
		area	134.05	2	<0.001	***	proximal > distal	0.25	5.37	<0.001	***
		nr parasites	0.24	1	0.621		proximal < central	-0.29	-6.17	<0.001	***
		species:area	14.37	12	0.278						
	C. furu	species	1.02	6	0.985		central vs. distal	0.05	0.60	0.820	
		area	21.48	2	<0.001	***	proximal < distal	-0.29	-3.60	0.001	***
		nr parasites	0.05	1	0.815		proximal < central	-0.33	-4.20	<0.001	***
		species:area	19.18	12	0.084	.					

· P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001; df, degrees of freedom.

were non-randomly distributed across vertical areas in 10 out of 13 and in longitudinal segments in 11 out of 14 infected host species (**Fig. S5.1** and **Table S5.4**).

The spatial distribution of *L. monodi* and *E. lamellifer* did not differ between host species (the only exception was the vertical distribution of *L. monodi*, **Fig. S5.1C**). In contrast, the spatial distribution of *Cichlidogyrus* spp. did differ between host species (**Fig. 5.3** and **Fig. S5.1**). These differences in distribution were observed at each level of spatial subdivision considered (gill arches, longitudinal segments and vertical areas; **Table 5.1**).

5.3.2. Non-random spatial distribution on fish gills: species of *Cichlidogyrus*

Sample size allowed statistical analysis only for the two most common species (*Cichlidogyrus nyanza* and *C. furu*). In line with the aforementioned pattern, species of *Cichlidogyrus* were non-randomly distributed on fish gills. *Cichlidogyrus nyanza* were non-randomly distributed regardless of the spatial subdivision considered (all 36 microhabitats, gill arches, longitudinal segments and vertical area); *C. furu* were non-randomly distributed among gill arches and vertical areas (**Table 5.2**, **Fig. S5.2**).

The two species of *Cichlidogyrus* had approximately similar distributions. Both were least abundant on the fourth gill arch and ventral segments, and most abundant in the central areas of the gills (for significant differences see **Table 5.2** and **Fig. S5.2**).

The non-random distributions of *Cichlidogyrus nyanza* and *C. furu* were also observed when testing each host species separately (**Fig. S5.3**, **Table S5.5**). *Cichlidogyrus nyanza* were non-randomly distributed across all gill microhabitats in four out of seven infected host species, across longitudinal segments (four out of seven) and across vertical areas (six out of seven). *Cichlidogyrus furu* were non-randomly distributed across vertical areas in three out of six infected host species.

The spatial distribution of both species of *Cichlidogyrus* differed between host species for the majority of the spatial divisions considered (except vertical areas for both species and longitudinal segment distribution for *Cichlidogyrus furu*; **Fig. S5.3**, **Table 5.2**).

5.3.3. Relationships between parasite taxa

To assess if parasite species are competing with or facilitating each other, we tested if the abundance of one parasite taxon was correlated with the abundance of another. After taking into account the differences in parasite abundance between host species, we observed that the abundance of both *Cichlidogyrus* spp. and of *L. monodi* were positively correlated with *E. lamellifer* (**Fig. 5.4**, **Table 5.3**). The positive direction of these relationships was observed also

when testing each host species separately, albeit not reaching statistical significance in most of them (**Table S5.6**). On the other hand, there was no positive association between *Cichlidogyrus* spp. and *L. monodi*. The abundance of glochidia was not associated with other parasites. Interspecific interactions between parasite genera did not differ between host species. Since some influential outliers (Cook's distance > 5) were identified in regressions of *L. monodi* and *E. lamellifer*, we repeated these analyses without those. This did not change the results (**Table S5.7**). Adding fish individual length as a fixed effect also did not change these results.

We also investigated interactions among species of *Cichlidogyrus*. Contrary to the pattern found at higher taxonomic level, all interactions between *Cichlidogyrus* species were negative (nine out of 10 relationships; there was one (non-significant) positive association; **Fig. 5.5**; **Table 5.3**). Differences between host species in species' interactions were not investigated due to the low sample size.

5.3.4. Reproductive success of copepods

The proportion of *L. monodi* carrying egg clutches was 77% and did not significantly differ between host species (33% ± S.D. 0.35 – 100% ± S.D. 0.00; **Table 5.4**). It also did not covary with individual fish length, capture water depth, CF, nor with the abundance of conspecifics or other parasites. The sample size of *E. lamellifer* was too low to perform statistical analyses (18 parasite individuals, 5.5% carrying egg sacs).

5.4. DISCUSSION

We investigated patterns of microhabitat specialisation, interspecific interactions and reproductive activity in gill parasites infecting sympatric cichlid species from Lake Victoria, to assess potential species specificity of the host-parasite relationships. We found that representatives of the two most abundant ectoparasite genera (*Cichlidogyrus* spp., *L. monodi*) and species of *Cichlidogyrus* (*C. nyanza*, *C. furu*) had a non-random spatial distribution on gills. *Cichlidogyrus* spp. and *L. monodi* occupied different microhabitat niches within the host, while the two species of *Cichlidogyrus* occupied similar microhabitats. In several cases, parasite spatial distributions differed between host species. Interactions among the different ectoparasite genera were synergistic, whereas among species of *Cichlidogyrus* they were antagonistic. Reproductive activity of the copepod *L. monodi* did not differ between host species and was not associated with the abundance of conspecific or heterospecific parasites.

5.4.1. Non-random spatial distribution on fish gills

We observed non-random microhabitat distributions for *Cichlidogyrus* spp. and for *L. monodi* that differed between these two parasite taxa. This suggests that they have adapted to different niches within the gills. The observed tendency for a non-random microhabitat distribution is consistent with previous findings in monogeneans (Morand et al., 2002; Bagge et al., 2005; Soylu et al., 2013) and copepods (Tsotetsi et al., 2004). Moreover, the actual distribution of monogeneans is consistent with previous studies (see below; Koskivaara & Valtonen, 1992; Bagge & Valtonen, 1996; Bagge et al., 2005; Blahoua et al., 2018; Blahoua et al., 2019).

Lamproglena monodi was most abundant in the central area along the gill filament, as previously observed in *Lamproglena clariae* (Tsotetsi et al., 2004), presumably promoting exposure of egg clutches to water flow. The rare copepod *E. lamellifer* had a random spatial distribution, suggesting that it may be a generalist parasite in terms of niche breadth, in addition to its documented broad host range (Scholz et al., 2018). However, the lack of a clear spatial pattern could also be due to its low abundance. At a comparably low abundance, a homogeneous microhabitat distribution was previously observed in *Ergasilus lizae* (Soylu et al., 2013). Further investigations in hosts with higher infection loads of *E. lamellifer* are needed to exclude an effect of low sample size on the observed pattern.

Cichlidogyrus spp. were less frequently found on the fourth gill arch, which is the smallest one. This is in line with previous findings on *Dactylogyrus*, reporting highest abundances on the largest arch in crucian carp (Bagge et al., 2005) and in roach (Koskivaara et al., 1992; Bagge & Valtonen, 1996) and low numbers on the fourth arch in two cichlid species, *Tylochromis jentinki* and *Tilapia zillii* (Blahoua et al., 2018; Blahoua et al., 2019). This may simply result from the available gill surface, providing space and resources to sustain fewer parasite individuals on the fourth arch and more on the first arch (Geets et al., 1997; El-Naggar & Reda, 2003; Madanire-Moyo et al., 2011). However, *L. monodi* (which is a much larger parasite) showed no differences between the first and fourth gill arches, suggesting that other mechanisms may explain the distribution of *Cichlidogyrus*. It cannot be explained by differences in water flow, as simulations demonstrated that water flow is similar along the first and fourth arch (Gutiérrez & Martorelli, 1999). However, water flow may influence the vertical distribution of *Cichlidogyrus* along the gill filament: it was less frequently found on the distal tip of gill filaments, where the water flow is maximal (Paling, 1968). This seems in contrast with previous studies, that found a higher abundance of other species of *Cichlidogyrus* in the distal area (Adou et al., 2017; Blahoua et al., 2019).

The extent of niche overlap between parasites may be linked to the direction of the correlations in parasite abundance. At the higher taxon level, parasites differed in spatial distributions and their abundances were positively correlated. This suggests a facilitating effect, in which reduced

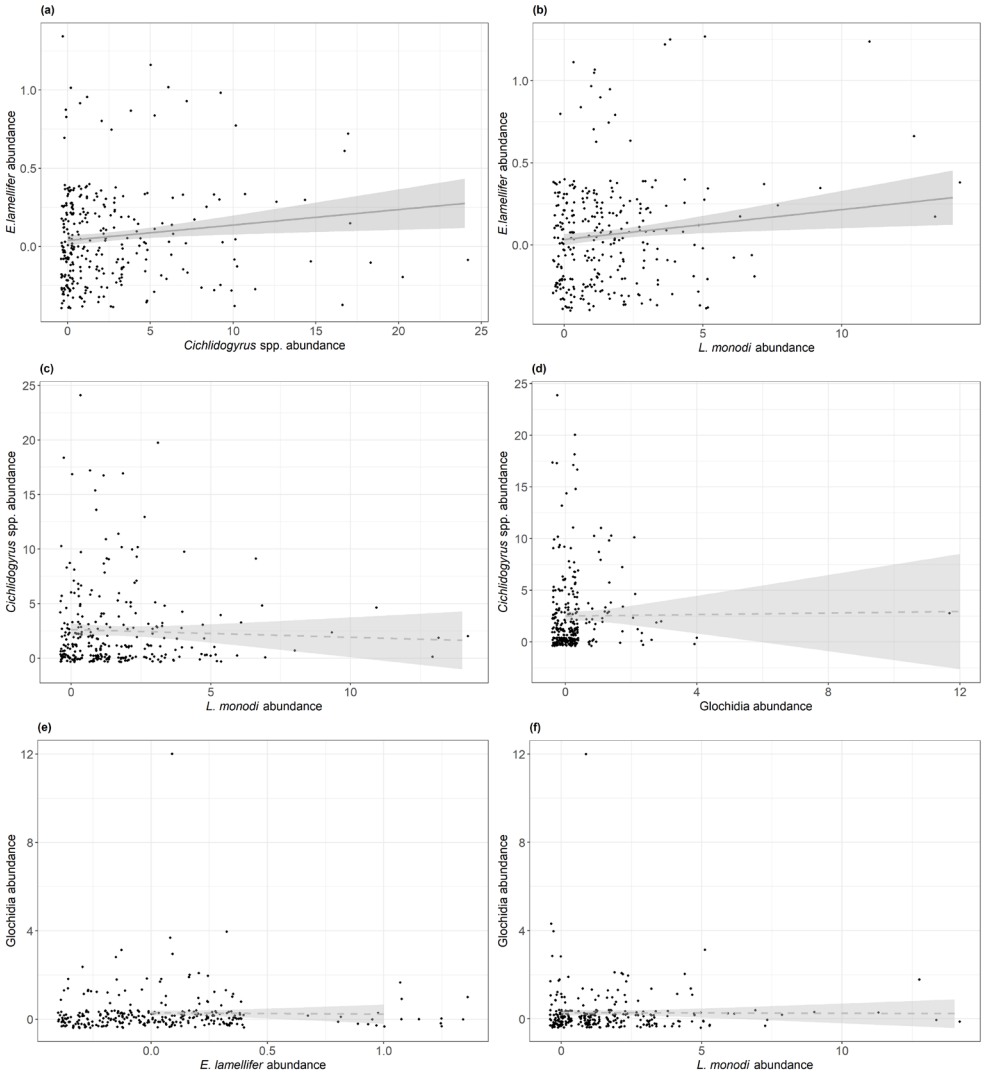


Figure 5.4
Significant relationships between the abundances of parasites of different genera infecting cichlids inhabiting Makobe Island. The abundance of *Ergasilus lamellifer* was positively associated (solid curves) with the abundance of **(a)** *Cichlidogyrus* spp. and of **(b)** *Lamproglena monodi*. The other parasites were not significantly correlated (dashed curves).

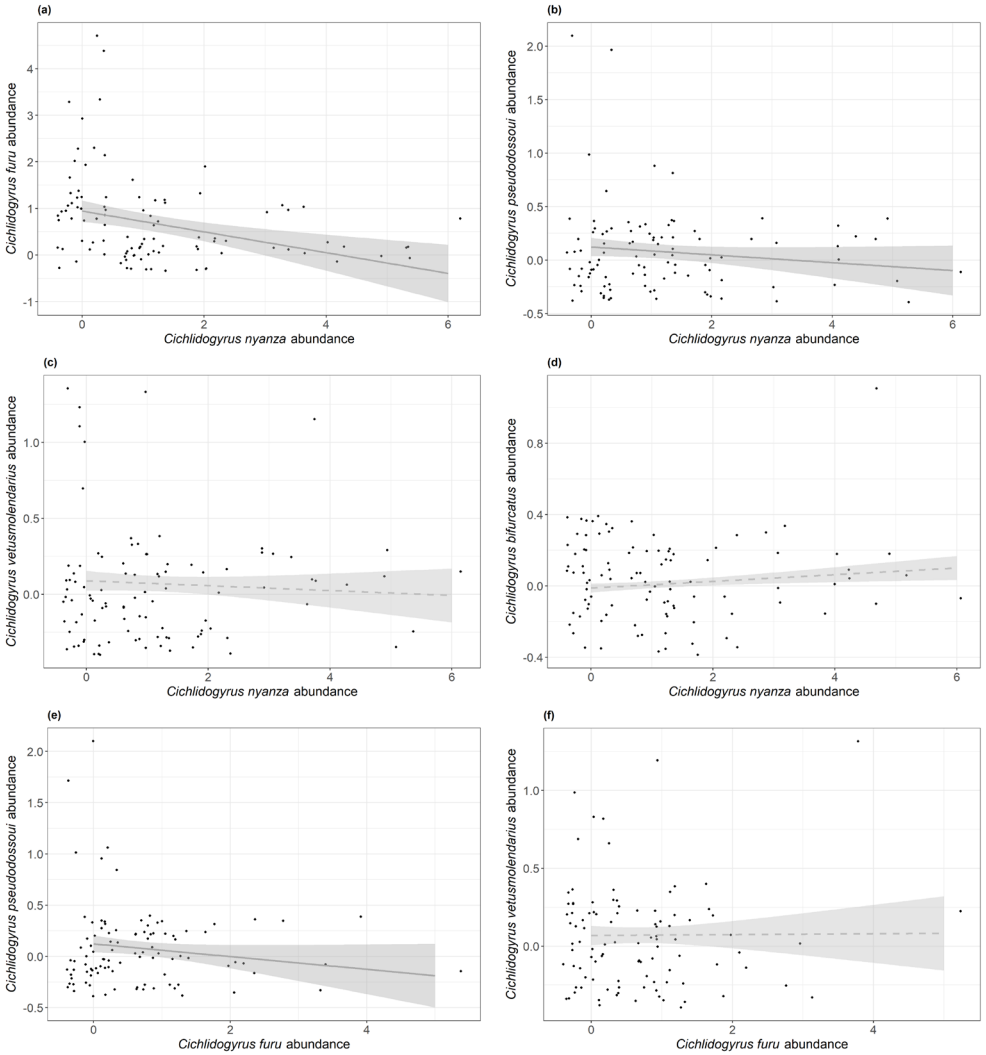


Figure 5.5
Significant relationships (solid curves) between the abundances of species of *Cichlidogyrus* infecting cichlids inhabiting Makobe Island. The abundance of *Cichlidogyrus nyanza* was negatively associated with abundance of **(a)** *Cichlidogyrus furu* and of **(b)** *Cichlidogyrus pseudodossoui*. The abundance of *Cichlidogyrus pseudodossoui* was also negatively associated with the abundance of **(e)** *Cichlidogyrus furu* and **(i)** *Cichlidogyrus vetusmolendarius*. The other species of *Cichlidogyrus* were not significantly correlated (dashed curves).

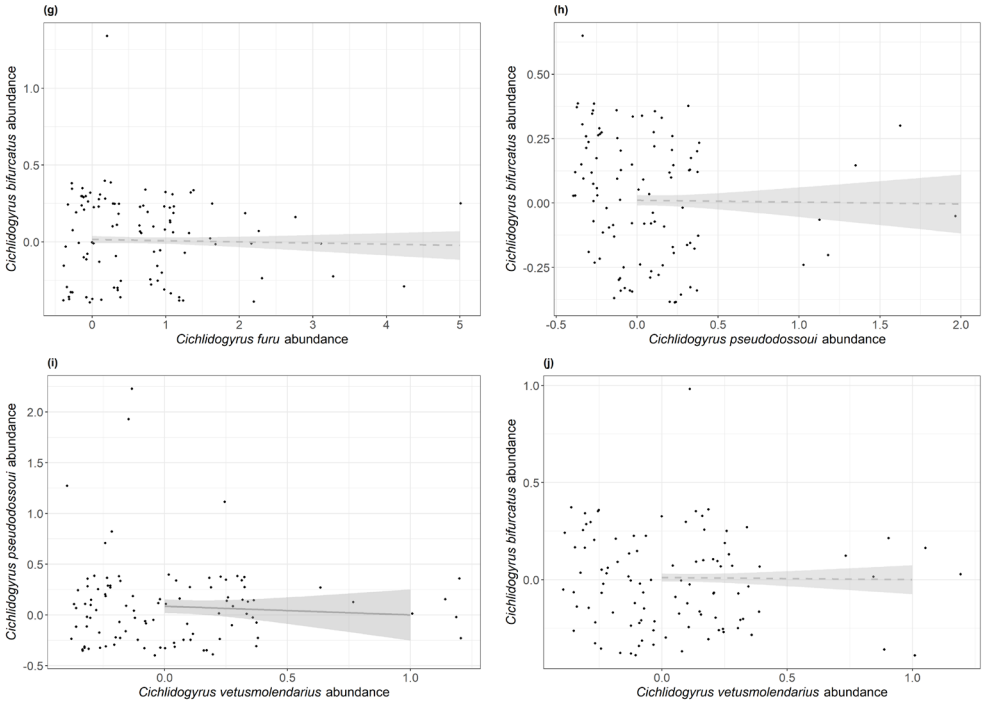


Figure 5.5 (continued)

Table 5.3
Interspecific abundance relationships between **(a)** parasite genera and **(b)** species of *Cichlidogyrus* infecting haplochromine cichlids at Makobe Island. The abundance of the focal parasite taxon was related with the abundance of another parasite taxon. The Minimum Adequate Models (MAM) were established by stepwise removal of non-significant variables from the starting model, which included host species, every parasite taxon and in (a) also the interaction term between host species and each parasite taxon (because of small sample size, the interaction was excluded in (b)).

(a)

Focal parasite	Fixed effects	LR	df	p		direction
Cichlidogyrus spp.	host species	175.33	11	<0.0001	***	
	E. lamellifer	8.09	1	0.004	**	+
Lamproglena	host species	53.07	11	<0.0001	***	
monodi	*E. lamellifer*	8.69	1	0.003	**	+
Ergasilus	*Cichlidogyrus*	5.36	1	0.021	*	+
lamellifer	*L. monodi*	5.26	1	0.022	*	+
Glochidia	1					

(b)

Focal *Cichlidogyrus*	Fixed effects	LR	df	p		direction
C. nyanza	host species	56.25	11	<0.0001	***	
	C. furu	11.66	1	0.001	***	-
C. furu	*C. nyanza*	23.36	1	<0.0001	***	-
	C. pseudodossoui	11.35	1	0.001	***	-
C. pseudodossoui	*C. nyanza*	20.97	1	<0.0001	***	-
	C. furu	25.04	1	<0.0001	***	-
	C. vetusmolendarius	7.30	1	0.007	**	-
C. vetusmolendarius	1					
C. bifurcatus	1					

· P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001; LR, likelihood ratios; df, degrees of freedom.

Table 5.4
Variation in the proportion of individuals of *Lamproglena monodi* carrying egg sacs in relation to host species identity, host individual length (SL), condition factor (CF), capture water depth, the abundance of conspecific and heterospecific parasites, fish preservation method (formalin vs. ethanol) and days elapsed between fish collection and parasite screening (time elapsed).

Fixed factors	LR	df	p
species	11.113	12	0.519
species:*Lamproglena monodi*	8.299	12	0.761
depth	0.690	1	0.406
time elapsed	0.425	1	0.514
glochidia	0.277	1	0.599
CF	0.235	1	0.628
preservation	0.136	1	0.712
SL	0.072	1	0.789
Lamproglena monodi	0.062	1	0.804
Ergasilus lamellifer	0.055	1	0.815
Cichlidogyrus spp.	0.015	1	0.901

LR, likelihood ratios; df, degrees of freedom.

host defences by one parasite lead to an increased infection with the other parasite taxon. Indeed both the copepods and monogeneans are known to induce host defences (copepods reviewed in Fast, 2014; monogeneans Zhi et al., 2018; Chen et al., 2019; Igeh & Avenant-Oldewage, 2020), implying that defence against one parasite could be at the expense of defence against another. On the other hand, within *Cichlidogyrus*, the analysed species had similar spatial distributions and their abundances were negatively correlated. Future studies may investigate if competition for space or other gill resources is indeed occurring among species of *Cichlidogyrus*.

5.4.2. Parasite spatial distributions in different host species

The non-random microhabitat distributions of *L. monodi* and *Cichlidogyrus* spp. (in particular the most common species, *C. nyanza*) were observed in most hosts. Such niche restriction may be a functional response to spatial variation in resource availability, or to competition between parasite taxa, even in the absence of a numerical response (i.e. reduction in the abundance, Thomson, 1980). However, since ectoparasites of cichlids from Lake Victoria are present in relatively low abundances (two to five-fold lower than in cichlids from Lake Tanganyika belonging to *Tropheus*, Raeymaekers et al., 2013; a hundred-fold lower than in Atlantic salmon in Norway, Jensen & Johnsen, 1992; Mo, 1992), we may speculate that competition among parasites is too weak to drive niche restriction (Rohde, 1979, 1991). Niche selection may be driven by other processes such as mating strategies. In parasites that mate on the host, such as monogeneans (Geets et al., 1997; Lo, 1999), a narrow niche increases the probability of contact with conspecifics and thereby facilitates mating (e.g. in crucian carp, Bagge et al., 2005; but see review by Morand et al., 2002). Alternatively, niche restriction may be the result of competition between parasite taxa in the evolutionary past (Poulin, 2007).

The spatial distribution of species of *Cichlidogyrus* differed between host species. This may indicate cryptic infection differences among host species, supporting specificity of the *Cichlidogyrus*-host interaction. This is in line with earlier observations that monogeneans with high host specificity have anchor sizes that match the gill arch size of their host species (Khang et al., 2016). Also, for *L. monodi* there are indications of host specificity; its spatial distribution along vertical areas differed between host species. If infection differences only accumulate after speciation, host species differences in the microhabitat distributions of their parasites might be more pronounced between more distantly related host species than between closely related species. We may then observe that spatial distribution patterns are more distinct between host species of different genera than within the same genus. Although not tested explicitly, we observed such a pattern for *Cichlidogyrus* spp., which were more abundant on the first gill arch in each of the three sampled species of *Pundamilia* than in other host genera, and for *L. monodi*, which were more abundant on the median segment (**Fig. 5.3**). Interestingly, this pattern is shared with *Mbipia mbipi* (a likely hybrid species between *Pundamilia* and *Mbipia*, Keller et al., 2013) and *Neochromis* sp. 'uniscuspid scraper' (a likely hybrid species between *Pundamilia* and

Neochromis, Seehausen et al. unpublished data). To properly address this, we would need a larger sample size of parasites, especially of representatives of *Cichlidogyrus* identified to species level.

5.4.3. Relationships between parasite taxa

Abundances of *Cichlidogyrus* spp. and *L. monodi* were positively associated with the abundance of *E. lamellifer* and vice-versa, whereas abundances of *Cichlidogyrus* spp. and *L. monodi* were not correlated. Positive associations may be explained in several ways. First, they may be true synergistic interactions, in which one parasite taxon increases the infection risk, disease severity and/or transmission rate of another parasite taxon (Hellard et al., 2015). Second, they may result from host populations sharing infection risk factors, leading to an increased co-occurrence even if parasites do not truly interact (Hellard et al., 2012). This seems unlikely, because positive associations also were observed in host species that differ in ecological specialisation (e.g. diet and water depth). Finally, we may speculate that the two copepod species (*L. monodi* and *E. lamellifer*) may facilitate each other because they may be antigenically similar enough to benefit from host susceptibility to the other copepod (Telfer et al., 2010) or from the immunomodulation induced by the other copepod (e.g. *Anaplasma* bacteria and cowpox virus in field voles, Telfer et al., 2010; HIV virus and hepatitis B virus in humans, Kellerman et al., 2003). However, host condition was not related to parasite load, as may be expected under natural conditions with relatively low parasite loads. It is unclear if such immunomodulation can happen even without affecting host condition, as the latter was not investigated in the aforementioned studies. The observation of positive associations does not exclude antagonistic interactions, as they may be present but outweighed by synergistic interactions.

In contrast to the positive correlations between parasite genera, abundances of species of *Cichlidogyrus* were negatively related. This may indicate that congeneric parasites are more prone to compete with each other, likely because they are more similar than non-congeners (and thus may have similar nutritional needs and attachment mode), as suggested by the similarity in spatial distribution between *Cichlidogyrus nyanza* and *C. furu*.

Since parasite community structure is thought to be mainly shaped by interspecific interactions (Poulin, 2001) we focused on those. Intraspecific interactions may be particularly relevant in monogenean communities, as they mate on the host and gills are far from being saturated (Rohde, 1979; Morand et al., 2002). On the other hand, copepods mate before attachment on the host and many of them cannot move after attachment, thus their spatial distribution is more likely shaped by interspecific interactions and/or by other factors (e.g. egg spreading).

5.4.4. Reproductive success of copepods

The reproductive success of *L. monodi* (measured as the proportion of copepod individuals carrying egg sacs) did not differ between host species, and was not correlated with the abundance of conspecifics nor the abundance of other ectoparasite taxa. This may support the low host specificity of *L. monodi*, which may be deduced from the observation that it is found in all cichlids sampled from Lake Victoria studied here and 48 African cichlid species in total (Karvonen et al., 2018; Scholz et al., 2018; **Gobbin et al., 2020b**).

5.5. CONCLUSION

Parasites had non-random gill microhabitat distributions, which differed between host species. This may indicate cryptic differences in the host-parasite interactions, potentially supporting parasite-mediated host differentiation - assuming that gill parasites exert pathogenic effects on their hosts. Microhabitat distribution may represent an important axis of differentiation between host species that is worth including in future studies.

Between and within parasite genera, we observed opposite patterns of niche overlap and abundance, suggesting that closely related parasites are more prone to compete with each other (probably due to similar resource requirements) whereas distantly related parasites tended to facilitate each other (possibly as opportunistic infections or through immunomodulation). Such parasite interactions did not differ between host species and thus do not constitute evidence for variation in host-parasite interactions.

5.6. ACKNOWLEDGEMENTS

This research was funded by the Swiss National Science Foundation and the University of Groningen (Ubbo Emmius Programme). Infrastructure was provided by the Natural History Museum in Lugano and Hasselt University (EMBRC Belgium - FWO project GOH3817N). We acknowledge Antoine Pariselle for help in identification of parasites belonging to *Cichlidogyrus*.

5.7. SUPPLEMENTARY MATERIAL

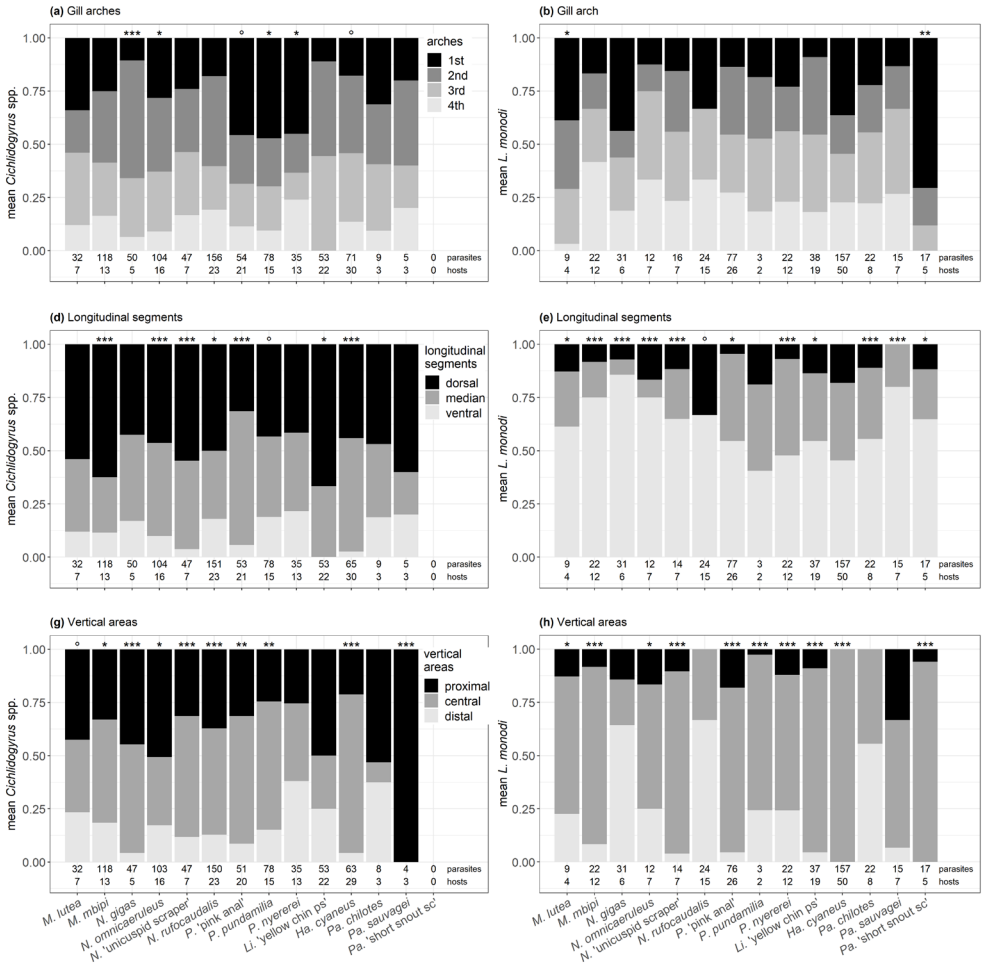


Figure S5.1
Within-host species spatial distributions on **(a-c)** gill arches, **(d-f)** longitudinal segments and **(g-i)** vertical areas of *Cichlidogyrus* spp. (left panels), *Lamproglena monodi* (central panels) and *Ergasilus lamellifer* (right panels) infecting cichlids inhabiting Makobe Island. Asterisks indicate a significant non-random spatial distribution within host species. The total number of parasites and of infected individuals per host species are reported.

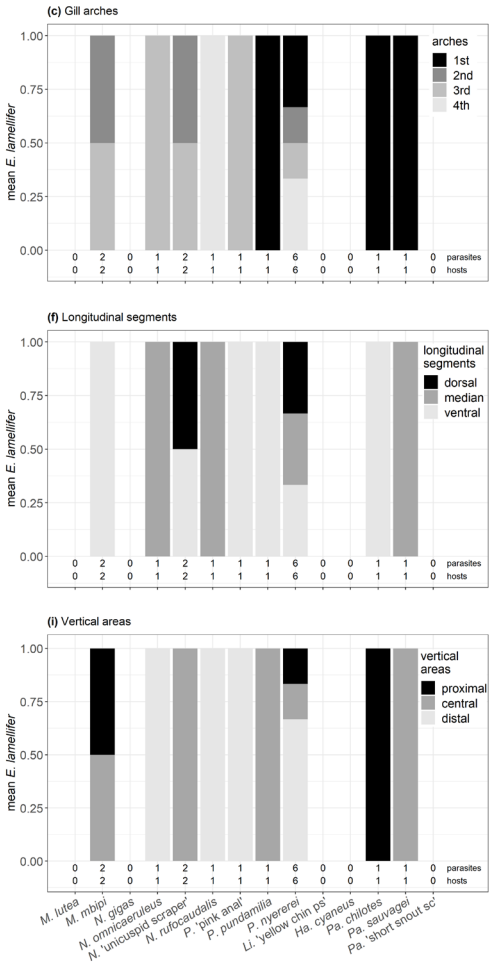


Figure S5.1 (continued)

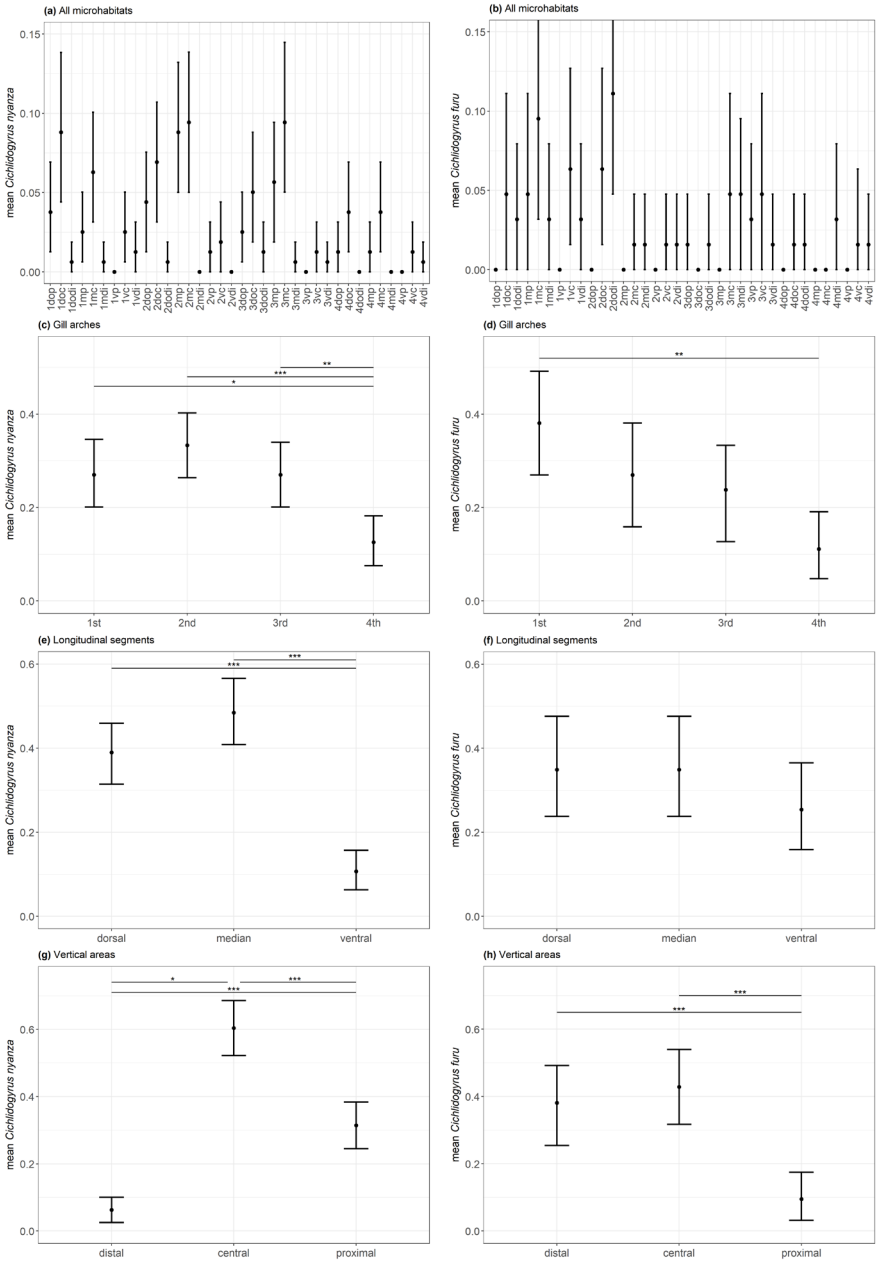


Figure S5.2
Spatial distributions of the two most common species of *Cichlidogyrus*, *C. nyanza* (left panels) and *C. furu* (right panels), infecting cichlids at Makobe Island. **(a-b)** gills 36 microhabitats, **(c-d)** gill arches, **(e-f)** longitudinal segments and **(g-h)** vertical areas. Asterisks indicate a significant difference in parasite spatial distribution between microhabitats (except in (a), where post-hoc tests were not performed).

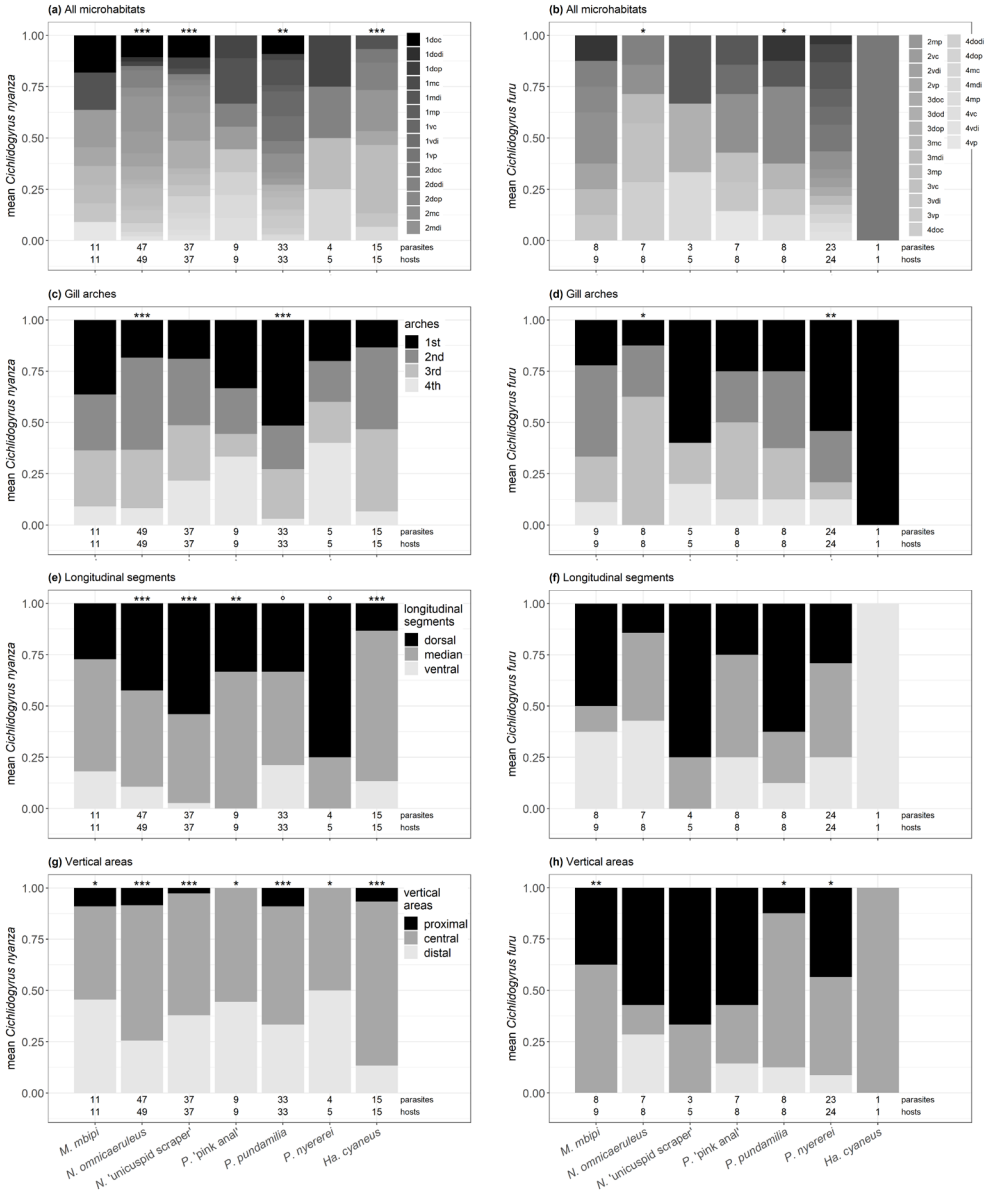


Figure S5.3
Within-host species spatial distributions on **(a)** gill arches, **(b)** longitudinal segments and **(c)** vertical areas of *Cichlidogyrus nyanza* (left panels) and *C. furu* (right panels) infecting cichlids inhabiting Makobe Island. Asterisks indicate a significant within-species non-random spatial distribution. The total number of parasites and of infected host individuals per species are reported.

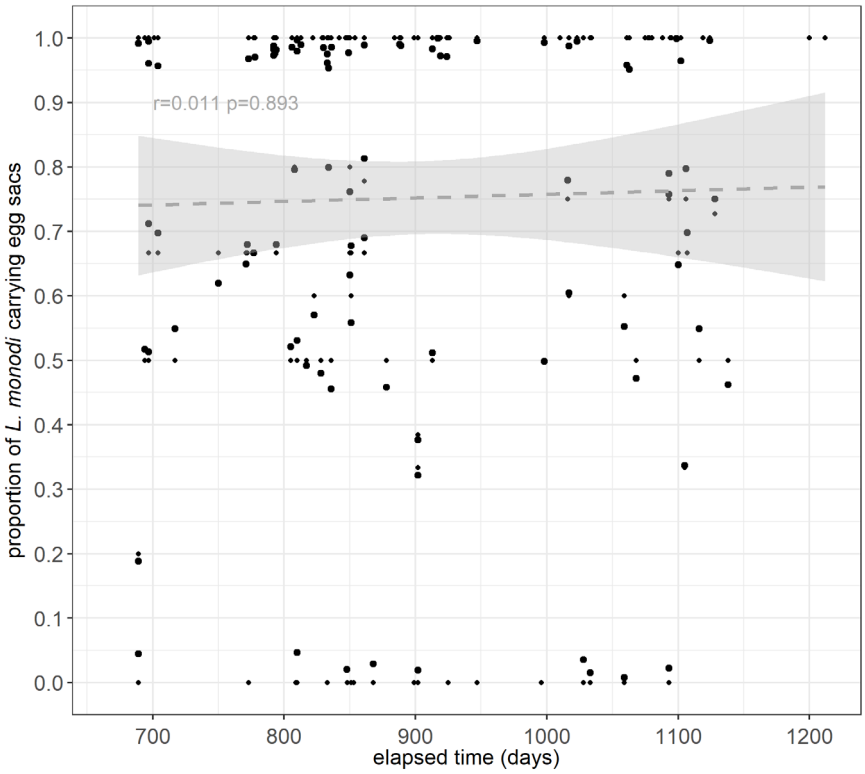


Figure S5.4
The elapsed time (days) between fish collection and parasite screening was not correlated with the proportion of *Lamproglena monodi* carrying egg sacs.

Table S5.1
Characteristics of host species (sampled in 2014 at Makobe Island, Lake Victoria): diet, number of fish individuals, water depth, SL standard length, weight, CF condition factor. The sample size of specimens of *Cichlidogyrus* identified to species level and the corresponding number of fish hosts are also reported.

			Depth (m)		SL (mm)		Weight (g)		CF		identified *Cichlidogyrus*	
Host species	Diet	N fish	mean	(min-max)	mean	(min-max)	mean	(min-max)	mean	(min-max)	N worms	N fish
M. lutea	algae	9	1.0	(1.0-1.0)	140.8	(135.9-149.9)	76.6	(67.1-82.5)	2.8	(2.47-3.08)	11	3
M. mbipi	algae	20	1.7	(1.0-2.5)	95.7	(83.2-113.1)	23.9	(18.2-33.7)	2.9	(2.54-3.72)	22	10
N. gigas	algae	8	1.2	(1.0-2.7)	115.0	(86.2-127.2)	17.2	(17.2-17.2)	2.8	(2.52-2.94)	15	3
N. omnicaeruleus	algae	36	4.4	(2.5-9.5)	92.2	(73.9-110.5)	22.1	(11.0-40.7)	2.8	(2.28-3.54)	25	13
N. sp. 'unicuspid scraper'	algae	32	13.2	(1.2-19.0)	96.7	(76.6-114.3)	25.5	(10.5-47.7)	2.7	(2.19-3.21)	23	20
N. rufocaudalis	algae	16	2.6	(0.7-3.5)	89.2	(61.3-100.0)	19.6	(6.1-25.8)	2.7	(2.41-3.08)	13	4
P. sp. 'pink anal'	plankton	18	9.9	(5.5-19.0)	91.8	(77.8-120.7)	23.9	(11.7-57.5)	2.8	(2.37-3.43)	15	6
P. pundamilia	insect	56	1.7	(0.5-16.0)	95.3	(52.1-128.7)	30.4	(3.6-70.0)	3.2	(2.50-3.76)	21	22
P. nyererei	plankton	78	10.2	(2.5-18.5)	81.0	(62.9-106.7)	15.4	(6.8-40.3)	2.7	(1.91-3.41)	34	22
Li. sp. 'yellow chin pseudonigricans'	insect	10	11.0	(9.0-19.0)	92.1	(79.7-112.9)	20.8	(12.3-47.0)	2.5	(2.23-3.26)	0	0
Ha. cyaneus	insect	14	2.7	(1.0-6.5)	100.2	(81.3-107.8)	23.6	(11.9-31.9)	2.3	(2.08-2.63)	16	5
Pa. chilotes	insect	13	13.8	(1.5-19.0)	106.9	(81.1-122.3)	32.1	(11.3-51.7)	2.5	(2.09-2.95)	5	4
Pa. sauvagei	insect	11	7.5	(3.5-14.0)	103.2	(93.7-115.3)	30.7	(11.3-44.8)	2.8	(1.06-3.42)	0	0
Pa. sp. 'short snout scraper'	algae	11	4.6	(3.0-6.0)	105.3	(93.5-115.5)	35.8	(22.0-43.1)	3.0	(2.70-3.29)	0	0

Table S5.2

Mean abundance (±SD) of ectoparasites *Cichlidogyrus* spp., *Lamproglena monodi* and *Ergasilus lamellifer* on gill arches (I, II, III, IV), on gill segments (dorsal, median, ventral) and on gill areas (proximal, central, distal) of infected cichlid species sampled at Makobe Island, Lake Victoria, in 2014. For copepod parasites, the proportion (±SD) of individuals carrying egg sacs is also reported.

		N fish		gill arches (4)								longitudinal segments (3)						vertical areas (3)						prop egg	
	Host species	tot	inf	arch I		arch II		arch III		arch IV		dorsal		median		ventral		proximal		central		distal			
Cichlidogyrus spp.	*M. lutea*	6	5	2.8	±5.5	1.7	±2.1	2.8	±2.9	1.0	±2.0	4.5	±3.8	2.8	±4.0	1.0	±0.9	3.3	±2.8	2.7	±2.5	1.8	±11.0		
	M. mbipi	16	16	1.6	±1.8	2.2	±1.4	1.6	±1.5	1.1	±1.6	4.1	±2.9	1.7	±1.8	0.8	±0.9	2.1	±2.0	3.1	±2.7	1.2	±19.0		
	N. gigas	8	7	0.6	±0.9	3.3	±2.5	1.6	±1.7	0.4	±0.7	2.5	±2.6	2.4	±2.3	1.0	±1.5	2.6	±1.7	3.0	±2.4	0.3	±2.0		
	N. omnicaeruleus	25	23	1.8	±2.9	2.2	±2.4	1.8	±1.9	0.6	±1.0	2.8	±3.4	2.6	±2.5	0.6	±1.2	3.0	±3.9	1.9	±2.4	1.0	±26.0		
	N. sp. 'unicuspid scraper'	30	21	0.4	±0.7	0.5	±1.1	0.5	±0.8	0.3	±0.7	1.0	±1.1	0.7	±1.0	0.1	±0.3	0.5	±0.9	1.0	±1.0	0.2	±6.0		
	N. rufocaudalis	15	15	0.9	±1.2	2.2	±2.1	1.1	±1.1	1.0	±1.7	2.6	±2.5	1.7	±1.5	0.9	±1.4	1.9	±1.6	2.6	±2.0	0.7	±10.0		
	P. sp. 'pink anal'	16	13	1.0	±1.0	0.5	±0.9	0.4	±0.6	0.3	±0.6	0.7	±1.0	1.4	±1.3	0.1	±0.3	0.7	±1.0	1.3	±1.4	0.2	±3.0		
	P. pundamilia	29	22	0.9	±1.2	0.4	±0.7	0.4	±0.6	0.2	±0.5	0.8	±0.8	0.7	±1.0	0.3	±0.7	0.5	±1.1	1.1	±1.4	0.3	±8.0		
	P. nyererei	58	30	0.6	±0.9	0.2	±0.6	0.2	±0.4	0.3	±0.8	0.5	±0.9	0.4	±0.8	0.2	±0.5	0.3	±0.7	0.4	±0.8	0.4	±24.0		
	Li. sp. 'yellow chin pseudonigricans'	9	3	0.1	±0.3	0.4	±0.9	0.4	±1.0	0.0	±0.0	0.7	±1.1	0.3	±0.7	0.0	±0.0	0.4	±0.9	0.2	±0.4	0.2	±2.0		
	Ha. cyaneus	14	13	1.5	±1.8	3.1	±2.3	2.7	±2.9	1.1	±2.0	3.7	±2.8	4.5	±3.7	0.2	±0.6	1.8	±1.9	6.3	±4.3	0.4	±5.0		
	Pa. chilotes	9	7	1.1	±2.3	1.0	±2.7	1.1	±2.3	0.3	±0.7	1.7	±4.3	1.2	±1.6	0.7	±2.0	1.9	±4.2	0.3	±0.5	1.3	±12.0		
	Pa. sauvagei	8	3	0.1	±0.4	0.3	±0.5	0.1	±0.4	0.1	±0.4	0.4	±0.7	0.1	±0.4	0.1	±0.4	0.5	±0.8	0.0	±0.0	0.0	±0.0		
	Pa. sp. 'short snout scraper'	5	0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0		
L.amproglena monodi	*M. lutea*	6	6	2.0	±12	1.7	±1.6	1.3	±1.5	0.2	±0.4	0.7	±0.8	1.3	±2.4	3.2	±1.9	0.7	±1.2	3.3	±2.8	1.2	±1.2	0.9	±1.0
	M. mbipi	16	7	0.1	±2.0	0.1	±0.3	0.2	±0.4	0.3	±0.6	0.1	±0.3	0.1	±0.3	0.6	±0.7	0.1	±0.3	0.6	±0.9	0.1	±0.3	0.6	±0.5
	N. gigas	8	7	0.9	±7.0	0.3	±0.5	0.5	±0.5	0.4	±0.7	0.1	±0.4	0.1	±0.4	1.5	±1.1	0.3	±0.5	0.4	±0.7	1.1	±1.1	0.9	±1.0
	N. omnicaeruleus	25	15	0.1	±3.0	0.1	±0.3	0.4	±0.7	0.3	±0.6	0.2	±0.5	0.1	±0.3	0.7	±1.0	0.2	±0.4	0.6	±0.8	0.2	±0.5	0.5	±0.5
	N. sp. 'unicuspid scraper'	30	26	0.4	±12.0	0.7	±0.8	0.8	±1.3	0.6	±0.6	0.3	±0.7	0.6	±0.8	1.7	±2.0	0.3	±0.8	2.2	±2.6	0.1	±0.3	0.7	±1.0
	N. rufocaudalis	15	2	0.1	±1.0	0.0	±0.0	0.1	±0.3	0.1	±0.3	0.1	±0.3	0.0	±0.0	0.1	±0.4	0.0	±0.0	0.1	±0.3	0.1	±0.5	0.3	±0.3
	P. sp. 'pink anal'	16	12	0.2	±3.0	0.4	±0.7	0.4	±0.6	0.4	±0.8	0.1	±0.3	0.6	±0.8	0.8	±0.9	0.3	±0.5	1.1	±1.2	0.1	±0.3	0.8	±1.0
	P. pundamilia	29	19	0.2	±7.0	0.4	±0.6	0.5	±0.6	0.2	±0.5	0.2	±0.5	0.5	±1.0	0.5	±0.8	0.0	±0.2	0.9	±1.2	0.3	±0.5	0.8	±1.0
	P. nyererei	58	50	0.6	±36.0	0.6	±1.1	0.9	±1.0	0.6	±0.8	0.2	±0.4	1.2	±1.6	1.3	±1.5	0.3	±0.7	1.7	±2.3	0.7	±1.2	0.8	±1.0
	Li. sp. 'yellow chin pseudonigricans'	9	8	0.2	±2.0	0.9	±1.1	0.9	±0.8	0.4	±0.7	0.3	±0.7	0.8	±1.1	1.3	±0.9	0.2	±0.4	2.1	±1.9	0.1	±0.3	0.7	±0.8
	Ha. cyaneus	14	12	0.6	±8.0	0.3	±0.5	0.4	±0.6	0.4	±0.6	0.3	±0.5	0.6	±0.7	0.7	±1.1	0.0	±0.0	1.6	±1.1	0.0	±0.0	0.9	±1.0
	Pa. chilotes	9	4	0.2	±2.0	0.2	±0.4	0.3	±1.0	0.2	±0.7	0.1	±0.3	0.3	±0.7	0.6	±0.7	0.0	0.00	0.4	±0.5	0.6	±1.3	1.0	±1.0
	Pa. sauvagei	8	7	0.3	±2.0	0.4	±0.7	0.8	±0.9	0.5	±0.5	0.0	±0.0	0.4	±0.7	1.5	±1.2	0.6	±1.4	1.1	±1.1	0.1	±0.4	0.0	±0.0
	Pa. sp. 'short snout scraper'	5	5	2.4	±12.0	0.6	±0.6	0.4	±0.9	0.0	±0.0	0.4	±0.9	0.8	±0.8	2.2	±0.8	0.2	±0.5	3.2	±1.6	0.0	±0.0	0.9	±1.0

Table S5.2 (continued)

		N fish		gill arches (4)								longitudinal segments (3)						vertical areas (3)						prop egg	
	Host species	tot	inf	arch I		arch II		arch III		arch IV		dorsal		median		ventral		proximal		central		distal			
Ergasilus lamellifer	*M. lutea*	6	0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
	M. mbipi	16	2	0.0	±0.0	0.1	±0.3	0.1	±0.3	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.1	±0.3	0.1	±0.3	0.0	±0.0	0.0	±0.0
	N. gigas	8	0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
	N. omnicaeruleus	25	1	0.0	±0.0	0.0	±0.0	0.0	±0.2	0.0	±0.0	0.0	±0.0	0.0	±0.2	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.2	0.0	±0.0
	N. sp. 'unicuspid scraper'	30	2	0.0	±0.0	0.0	±0.2	0.0	±0.2	0.0	±0.0	0.0	±0.2	0.0	±0.0	0.0	±0.2	0.0	±0.0	0.1	±0.3	0.0	±0.0	0.0	±0.0
	N. rufocaudalis	15	1	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.0	±0.0	0.1	±0.3	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.0	±0.0
	P. sp. 'pink anal'	16	1	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.0	±0.0
	P. pundamilia	29	1	0.0	±0.2	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.2	0.0	±0.0	0.0	±0.2	0.0	±0.0	0.0	±0.0
	P. nyererei	58	6	0.0	±0.2	0.0	±0.1	0.0	±0.1	0.0	±0.2	0.0	±0.2	0.0	±0.2	0.0	±0.2	0.0	±0.1	0.0	±0.1	0.1	±0.3	0.2	±0.4
	Li. sp. 'yellow chin pseudonigricans'	9	0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
	Ha. cyaneus	14	0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0
	Pa. chilotes	9	1	0.1	±0.3	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.3	0.1	±0.3	0.0	±0.0	0.0	±0.0	0.0	±0.0
	Pa. sauvagei	8	1	0.1	±0.4	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.1	±0.4	0.0	±0.0	0.0	±0.0	0.1	±0.4	0.0	±0.0	0.0	±0.0
	Pa. sp. 'short snout scraper'	5	0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0	0.0	±0.0

Table S5.3
Differences in fish measurements (length, weight) and parasite intensities (*Cichlidogyrus* spp., *Lamproglena monodi*, *Ergasilus lamellifer*) between host preservation methods (formalin vs. ethanol) and according to the time elapsed between host death and sampling. The reported contribution of each fixed effect was assessed through ANOVA.

model	LR	df	p	
SL				
host species	248.62	13	<0.0001	***
time elapsed	0.58	1	0.448	
preserv	2.83	1	0.092	
time elapsed : preserv	0.18	1	0.674	
weight				
host species	698.83	13	<0.0001	***
time elapsed	1.65	1	0.199	
preserv	41.01	1	<0.0001	***
time elapsed : preserv	1.68	1	0.195	
Cichlidogyrus spp. intensity				
host species	113.43	12	<0.0001	***
time elapsed	3.55	1	0.059	.
preserv	10.78	1	0.001	**
time elapsed : preserv	3.08	1	0.079	
Lamproglena monodi intensity				
host species	35.36	13	0.001	***
time elapsed	0.29	1	0.591	
preserv	0.05	1	0.827	
time elapsed : preserv	0.33	1	0.565	
Ergasilus lamellifer intensity				
host species	0.00	9	1.000	
time elapsed	0.00	1	1.000	
preserv	0.00	1	1.000	
time elapsed : preserv	0.00	1	1.000	

. P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001;LR, likelihood ratios; df, degrees of freedom.

Table S5.4
Spatial distribution on fish gills for each host species (all 36 microhabitats, gill arches, segments and areas) of parasite taxa infecting cichlids at Makobe Island. Significance level was corrected for pseudo-replication using Benjamini-Hochberg. *Cichlidogyrus* spp. and *Lamproglena monodi* were non-randomly located on the gills of most host species.

			all microhabitats (36)				gill arches (4)			
	Host	**N par**	**Chi sq**	**df**	**p**		**Chi sq**	**df**	**p**	
Cichlidogyrus spp.	*M. lutea*	47	41.92		0.283		1.35	3	0.778	
	M. mbipi	103	95.48		<0.001	***	4.66	3	0.323	
	N. gigas	47	96.16		<0.001	***	19.51	3	0.003	**
	N. omnicaeruleus	150	98.42		<0.001	***	11.09	3	0.037	*
	N. sp. 'unicuspid scraper'	51	59.05		0.012	*	1.75	3	0.739	
	N. rufocaudalis	78	66.98		0.002	**	7.74	3	0.096	
	P. sp. 'pink anal'	35	96.80		<0.001	***	9.83	3	0.052	.
	P. pundamilia	53	57.55		0.015	*	13.67	3	0.015	*
	P. nyererei	63	38.04		0.433		15.23	3	0.011	*
	Li. sp. 'yellow chin pseudonigricans'	8	30.67		0.734		4.25	3	0.334	
	Ha. cyaneus	118	73.42		<0.001	***	8.82	3	0.069	.
	Pa. chilotes	32	11.83		0.999		4.04	3	0.334	
	Pa. sauvagei	4	32.00		0.725		0.75	3	0.861	
	Pa. sp. 'short snout scraper'	0	NA		NA		NA	3	NA	
Lamproglena monodi	*M. lutea*	31	83.11	35	<0.001	***	13.44	3	0.026	*
	M. mbipi	12	54.00	35	0.025	*	2.57	3	0.719	
	N. gigas	14	71.70	35	0.001	***	5.42	3	0.335	
	N. omnicaeruleus	24	57.00	35	0.015	*	7.94	3	0.221	
	N. sp. 'unicuspid scraper'	76	175.86	35	<0.001	***	4.33	3	0.456	
	N. rufocaudalis	3	33.00	35	0.608		1.00	3	0.863	
	P. sp. 'pink anal'	22	57.02	35	0.015	*	1.42	3	0.816	
	P. pundamilia	37	55.99	35	0.017	*	3.46	3	0.571	
	P. nyererei	157	153.58	35	<0.001	***	7.06	3	0.245	
	Li. sp. 'yellow chin pseudonigricans'	22	89.25	35	<0.001	***	6.10	3	0.300	
	Ha. cyaneus	22	84.60	35	<0.001	***	1.72	3	0.804	
	Pa. chilotes	9	28.89	35	0.757		0.19	3	0.979	
	Pa. sauvagei	15	60.38	35	0.009	**	2.19	3	0.748	
	Pa. sp. 'short snout scraper'	17	117.20	35	<0.001	***	17.39	3	0.008	**
Ergasilus lamellifer	*M. lutea*	0	NA	35	NA		NA	3	NA	
	M. mbipi	2	11.56	35	1.000		2.00	3	0.644	
	N. gigas	0	NA	35	NA		NA	3	NA	
	N. omnicaeruleus	1	7.17	35	1.000		2.77	3	0.642	
	N. sp. 'unicuspid scraper'	2	7.17	35	1.000		2.00	3	0.644	
	N. rufocaudalis	1	7.17	35	1.000		2.77	3	0.642	
	P. sp. 'pink anal'	1	7.17	35	1.000		2.77	3	0.642	
	P. pundamilia	1	7.17	35	1.000		2.77	3	0.642	
	P. nyererei	6	30.00	35	0.980		0.77	3	0.857	
	Li. sp. 'yellow chin pseudonigricans'	0	NA	35	NA		NA	3	NA	
	Ha. cyaneus	0	NA	35	NA		NA	3	NA	
	Pa. chilotes	1	7.17	35	1.000		2.77	3	0.642	
	Pa. sauvagei	1	7.17	35	1.000		2.77	3	0.642	
	Pa. sp. 'short snout scraper'	0	NA	35	NA		NA	3	NA	

. P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001;df, degrees of freedom; NA, not available.

Table S5.4 (continued)

longitudinal segments (3)			vertical areas (3)		
Chi sq	df	p	Chi sq	df	p
5.47	2	0.094	6.18	2	0.059 .
23.58	2	<0.001 ***	8.78	2	0.020 *
2.68	2	0.310	16.97	2	0.001 ***
17.62	2	0.000 ***	7.65	2	0.032 *
23.51	2	0.000 ***	17.09	2	0.001 ***
7.88	2	0.036 *	22.26	2	<0.001 ***
20.70	2	<0.001 ***	13.16	2	0.003 **
6.07	2	0.078 .	11.22	2	0.007 **
4.11	2	0.166	1.55	2	0.499
9.00	2	0.024 *	0.89	2	0.641
29.50	2	0.000 ***	50.94	2	<0.001 ***
1.86	2	0.428	2.50	2	0.338
1.60	2	0.449	32.01	2	<0.001 ***
NA	2	NA	NA	2	NA
8.42	2	0.021 *	9.31	2	0.015 *
26.31	2	<0.001 ***	25.58	2	<0.001 ***
29.04	2	<0.001 ***	5.86	2	0.068 .
20.20	2	<0.001 ***	8.98	2	0.016 *
27.78	2	<0.001 ***	34.80	2	<0.001 ***
6.01	2	0.058 .	1.20	2	0.549
9.28	2	0.015 *	19.41	2	<0.001 ***
2.71	2	0.278	27.76	2	<0.001 ***
33.76	2	0.000 ***	28.14	2	<0.001 ***
7.98	2	0.024 *	20.47	2	<0.001 ***
2.25	2	0.325	91.82	2	<0.001 ***
18.00	2	<0.001 ***	2.93	2	0.249
18.00	2	<0.001 ***	3.79	2	0.175
12.47	2	0.015 *	33.24	2	<0.001 ***
NA	2	NA	NA	2	NA
4.39	2	0.428	1.00	2	0.607
NA	2	NA	NA	2	NA
2.20	2	0.428	2.20	2	0.375
1.00	2	0.682	4.39	2	0.375
2.20	2	0.428	2.20	2	0.375
2.20	2	0.428	2.20	2	0.375
2.20	2	0.428	2.20	2	0.375
0.00	2	1.000	5.00	2	0.375
NA	2	NA	NA	2	NA
NA	2	NA	NA	2	NA
2.20	2	0.428	2.20	2	0.375
2.20	2	0.428	2.20	2	0.375
NA	2	NA	NA	2	NA

Table S5.5

Non-random spatial distribution on fish gills for each host species (all 36 microhabitats, gill arches, gill segments and gill area) of the two most common species of *Cichlidogyrus* infecting cichlids at Makobe Island. Significance level was corrected for pseudo-replication using Benjamini-Hochberg. *Cichlidogyrus nyanza* was non-randomly located on the gills of most host species.

			all microhabitats (36)				gill arches (4)				longitudinal segments (3)				vertical areas (3)			
	Host	N par	Chi sq	df	p		Chi sq	df	p		Chi sq	df	p		Chi sq	df	p	
Cichlidogyrus nyanza	*M. mbipi*	13	47.21	35	0.114		2.21	3	0.773		3.76	2	0.152		7.15	2	0.033	*
	N. omnicaeruleus	49	104.33	35	0.000	***	20.90	3	0.000	***	17.84	2	0.000	***	46.86	2	0.000	***
	P. pundamilia	35	65.25	35	0.000	***	24.40	3	0.773		5.43	2	0.000	***	22.36	2	0.000	***
	P. nyererei	8	68.21	35	0.614		0.67	3	0.881		5.87	2	0.074	.	5.87	2	0.053	.
	N. sp. 'unicuspid scraper'	37	75.18	35	0.492		2.10	3	0.791		30.44	2	0.004	**	35.27	2	0.021	*
	P. pink anal	9	36.00	35	0.003	**	1.52	3	0.000	***	12.00	2	0.077	.	8.40	2	0.000	***
	Ha. cyaneus	15	101.77	35	0.000	***	7.85	3	0.115		23.63	2	0.000	***	40.90	2	0.000	***
Cichlidogyrus furu	*M. mbipi*	12	38.19	35	0.326		2.71	3	0.657		3.67	2	0.320		13.13	2	0.008	**
	N. omnicaeruleus	8	53.98	35	0.064	.	11.53	3	0.028	*	1.51	2	0.497		2.80	2	0.296	
	P. pundamilia	9	61.60	35	0.565		1.52	3	0.286		4.17	2	0.320		8.40	2	0.301	
	P. nyererei	24	46.05	35	0.200		19.02	3	0.002	**	2.61	2	0.407		10.27	2	0.018	*
	N. sp. 'unicuspid scraper'	5	33.00	35	0.338		5.43	3	0.749		5.60	2	0.497		2.40	2	0.296	
	P. pink anal	8	40.96	35	0.022	*	1.22	3	0.749		1.40	2	0.320		2.80	2	0.030	*
	Ha. cyaneus	1			NA				NA				NA				NA	

. P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001;df, degrees of freedom; NA, not available.

Table S5.6

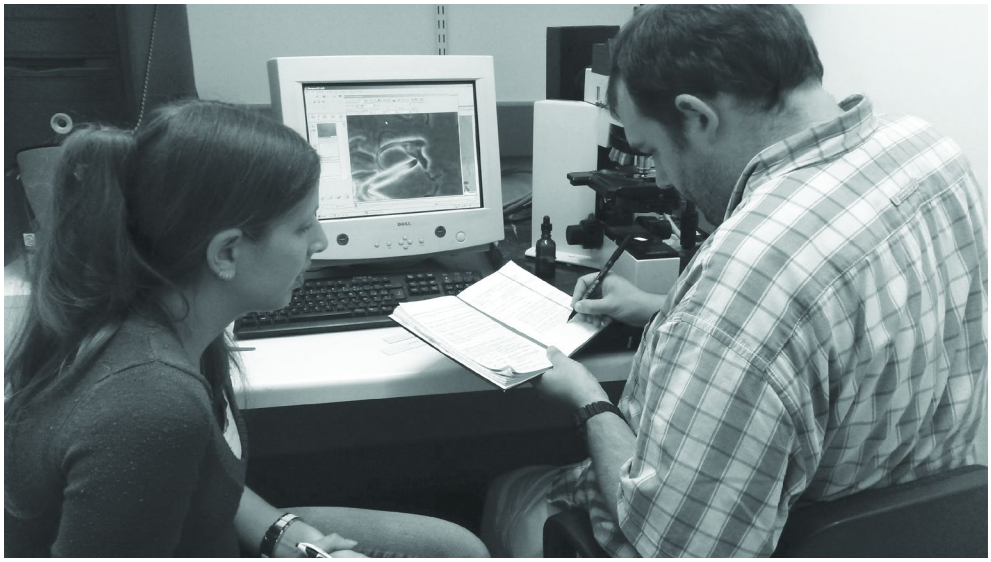
Relationships between the abundances of different ectoparasite genera for each cichlid host species sampled at Makobe Island. Although not always statistically significant, most relationships are positive.

Interaction	Host species	LR	df	p		direction	Interaction	LR	df	p		direction
Cichlidogyrus spp. vs. *Ergasilus lamellifer*	*M. lutea*			NA			*Ergasilus lamellifer* vs. *Cichlidogyrus* spp.			NA		
	M. mbipi	1.26	1	0.525		+		1.61	1	0.410		+
	N. gigas			NA						NA		
	N. omnicaeruleus	0.62	1	0.539		+		0.57	1	0.561		+
	N. sp. 'unicuspid scraper'	7.28	1	0.023	*	+		6.93	1	0.085	.	+
	N. rufocaudalis	13.97	1	0.002	**	+		5.55	1	0.093		+
	P. sp. 'pink anal'	0.76	1	0.539		+		0.90	1	0.561		+
	P. pundamilia	2.97	1	0.212		+		3.72	1	0.134		+
	P. nyererei	0.00	1	0.965		-		0.00	1	0.966		-
	Li. sp. 'yellow chin pseudonigricans'	0.84	1	0.539		-		0.71	1	0.561		-
	Ha. cyaneus			NA						NA		
	Pa. chilotes	0.16	1	0.764		+		0.21	1	0.717		+
	Pa. sauvagei	9.04	1	0.013	*	+		4.80	1	0.095		-
	Pa. sp. 'short snout scraper'			NA						NA		
Lamproglena monodi vs. *Ergasilus lamellifer*	*M. lutea*			NA			*Ergasilus lamellifer* vs. *Lamproglena monodi*	0.00	1	1.000		
	M. mbipi	4.13	1	0.116		-		6.47	1	0.092		-
	N. gigas			NA				0.00	1	1.000		
	N. omnicaeruleus	0.34	1	0.894		+		0.37	1	1.000		+
	N. sp. 'unicuspid scraper'	0.07	1	0.919		+		0.08	1	1.000		+
	N. rufocaudalis	0.99	1	0.702		+		2.37	1	0.432		+
	P. sp. 'pink anal'	0.03	1	0.919		+		0.03	1	1.000		+
	P. pundamilia	0.32	1	0.894		+		0.35	1	1.000		+
	P. nyererei	12.37	1	0.002	**	+		7.29	1	0.048	*	+
	Li. sp. 'yellow chin pseudonigricans'	0.01	1	0.919		+		0.01	1	1.000		+
	Ha. cyaneus			NA				0.00	1	1.000		
	Pa. chilotes	7.21	1	0.027	*	+		$5.4\ 10^{9}$	1	0.000	***	+
	Pa. sauvagei	0.11	1	0.919		-		0.11	1	1.000		-
	Pa. sp. 'short snout scraper'			NA				0.00	1	1.000		

Table S5.7
Interspecific abundance relationships between parasite genera infecting haplochromine cichlids at Makobe Island, after the removal of influential outliers (Cook's distance >0.5) from the regressions of *Lamproglena monodi* and *Ergasilus lamellifer*. The abundance of the focal parasite taxon was related with the abundance of another parasite taxon. The Minimum Adequate Models (MAM) were established by stepwise removal of non-significant variables from the starting model, which included host species, every parasite taxon and the interaction term between host species and each parasite taxon.

Focal parasite	Fixed effects	LR	df	p		direction
Cichlidogyrus	host species	175.33	11	<0.0001	***	
spp.	*E. lamellifer*	8.09	1	0.004	**	+
Lamproglena	host species	51.12	11	<0.0001	***	
monodi	*E. lamellifer*	8.56	1	0.003	**	+
Ergasilus	*Cichlidogyrus*	5.61	1	0.018	*	+
lamellifer	*L. monodi*	5.10	1	0.024	*	+
Glochidia	1					

. P≤0.1; * P≤0.05; ** P≤0.01; *** P≤0.001;df, degrees of freedom.



6

Four new species of *Cichlidogyrus* (Platyhelminthes, Monogenea, Dactylogyridae) from Lake Victoria haplochromine cichlid fishes, with the redescription of *C. bifurcatus* and *C. longipenis*

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7

7

General synthesis

Tiziana P Gobbin

Mechanisms of speciation have been investigated in many animal and plant taxa by countless studies. Many of them focused on ecological speciation, namely on the role of resource competition and interspecific interactions (as prey-predator and host-parasite) in driving species divergence (Nosil, 2012). Parasites may be an important source of ecological selection, as they can have strong effects on their hosts (e.g. negatively affecting host growth, reproduction and/or behaviour). Locally different parasite infection may drive adaptive host population divergence that may promote reproductive isolation between host populations and ultimately speciation. Studies on the role of parasites in host diversification have begun to accumulate (Greischar & Koskella, 2007; Eizaguirre et al., 2011; Eizaguirre et al., 2012a; Stutz et al., 2014; Feulner et al., 2015; Karvonen et al., 2015). However, it is still unclear at what stage of the speciation process parasite-mediated divergent selection acts; and to what extent it actually contributes to speciation, especially in the context of adaptive radiation (Vanhove & Huyse, 2015; El Nagar & MacColl, 2016). In this thesis, I investigated these aspects by studying the role of parasites in the diversification of cichlid species from Lake Victoria.

In the following pages, I summarise the main findings of previous chapters of this thesis (**Table 7.1**) and discuss their implications for the understanding of parasite-mediated divergence and speciation in natural hosts and particularly in African cichlids. Since results differed according to the parasite infection level analysed – between parasites of higher taxonomic levels (hereafter referred to as parasite higher taxon level) and between species of *Cichlidogyrus* (hereafter referred to as *Cichlidogyrus* species level) – I present them separately. I also suggest directions for future research, for the cichlid model system and beyond.

7.1. THE ROLE OF PARASITES (AT HIGHER TAXON LEVEL) IN HOST DIVERSIFICATION

Temporally consistent differences in infection between host species – I tested two prerequisites for parasite-mediated speciation – namely species differences in infection and temporal consistency in the direction of such differences. To this end, I analysed parasite infection patterns (parasite prevalence, abundance and parasite community composition; see **Box I** for definitions) of closely related cichlid species living in sympatry in southern Lake Victoria. The number and diversity of parasites differed between reproductively isolated cichlid species (**chapters 2 and 3**). These contrasting infection patterns among host species were not unexpected, given the ecological diversity of Lake Victoria cichlids (Greenwood, 1981; Witte & van Oijen, 1990; Seehausen, 1996b; Bouton et al., 1997; Bouton et al., 1999.), but they were remarkable given the young age of the Lake Victoria radiation (14'600 years old; Johnson et al., 1996). Such species differences in infection patterns remained consistent after a period of four years. These findings suggest that parasite-mediated selection is indeed divergent in cichlids and

that its direction is maintained over time, possibly facilitating a role of parasites in host divergence.

In addition to species variation in parasite numbers and diversity, I also found another axis of species divergence in infection: parasite microhabitat distribution over the fish gills (based on the number of parasites found on each of 36 spatial subdivisions of the gills; **chapter 5**). Most parasite taxa had non-random microhabitat distributions on gills, which also differed between parasite species. In addition, microhabitat distribution patterns of *Cichlidogyrus* spp. and *L. monodi* were more distinct between host species of different genera than within the same genus. This host genus effect suggests that parasites differences accumulate after speciation. The non-random microhabitat distribution mostly differs between host species (**chapter 5**), suggesting it is unrelated to parasite interspecific relationships, but may rather result from other factors, such as egg spreading, tissue accessibility or enhancement of mating opportunities. In particular, mating facilitation seem to be important in monogeneans – as they are hermaphrodites with obligate cross-fertilization that reproduce in the host – whereas egg spreading seems to be important in copepods – as they mainly position themselves in the distal parts of the gills, exposing the eggs to water flow. Together, these findings may indicate host species-specificity in parasite niche selection and consequently in the host-parasite relationship, consistent with a role of parasites in host differentiation. Microhabitat distribution patterns of *Cichlidogyrus* spp. were largely based on many parasite individuals not identified at species level. Hence these patterns could result from host species harbouring different species of *Cichlidogyrus*, some of which have different microhabitat distributions (e.g. microhabitat distribution in *P. pundamilia* could be driven by a higher abundance of *Cichlidogyrus furu* than other species). To address this, it would be necessary to identify more specimens of the collected *Cichlidogyrus*.

In many copepod species, females have egg clutches appended to their body, allowing me to investigate whether their reproductive activity constitutes another axis of infection variation between host species. The proportion of female copepods carrying egg clutches was similar among all sampled wild host species (**chapter 5**) and among the two lab-reared species of *Pundamilia* and their interspecific hybrids (**chapter 4**). This suggests that reproductive activity of copepods is not influenced by host species identity nor by host ecology, hence it does not constitute an infection trait under divergent selection.

Table 7.1
Key findings presented in this thesis, at parasite higher taxon level (P) and at *Cichlidogyrus* species level (C), and whether they support or not a role of parasites in host diversification (* indicates support for sympatric diversification, NA indicates that the finding alone is not relevant in the context of parasite-mediated diversification).

Chapter	**Study system**, key findings		**Support**
2	**Community of sympatric cichlid species, belonging to three lineages (radiation, *A. alluaudi*, *Ps. multicolor*) and differing in ecological specialisations**		
	P:	Host species of a large community show temporally consistent infection differences.	yes
	C:	Infection pattern of species of *Cichlidogyrus* differs between host lineages, but not between closely related species within the radiating lineage.	no
	P, C:	Variation in infection between host species is not fully explained by differences in host ecology. Instead, the best predictor of infection was species identity.	yes
	P, C:	Lack of geographic pattern in infection profiles.	yes*
3	**Four pairs of closely related species/forms of *Pundamilia***		
	P:	Temporally consistent infection differences between already differentiated pairs of closely related host species.	yes
	P:	Infection divergence accumulates as host genetic differentiation increases.	yes
	C:	Infection divergence is unrelated to host genetic differentiation.	no
	P, C:	Variation in infection between forms is not fully explained by differences in host ecology alone. Instead, the best predictor of infection was population identity.	yes
	P, C:	Lack of geographic pattern in infection profiles.	yes*
4	**Community of sympatric cichlid species, belonging to the radiating lineage and differing in ecological specialisations**		
	P, C:	Non-random spatial distribution in the host gills.	partial
	P:	Parasite taxa differ in gill microhabitat distribution.	partial
	C:	Species of *Cichlidogyrus* have overlapping gill microhabitats.	NA
	P, C:	Parasite spatial distribution on gills differs between host species.	yes
	P, C:	Directions of parasite-parasite abundance relationships differ between taxonomical level (positive between parasite genera, negative within *Cichlidogyrus*), but do not differ between host species.	no
	-	Reproductive activity of *L. monodi* does no differ between host species.	no
5	**Wild and lab-bred representatives of two closely related species of *Pundamilia* and their lab-bred hybrids**		
	P:	Species differences in infection in the wild are not maintained in laboratory conditions.	no
	P:	No hybrid disadvantage in infection levels.	no
	-	Reproductive activity of *L. monodi* does no differ between host species.	no
6	**Species of *Cichlidogyrus* infecting cichlids of southern Lake Victoria**		
	C:	Formal description of four new species of *Cichlidogyrus* and re-description of other two species.	NA

What stage of the speciation process do parasites contribute? – The role of parasites in host speciation (if any) may range between two extremes. On one extreme, parasites may be crucial for the evolution of host reproductive isolation and ultimately for host divergence (driver role). On the other extreme, parasites may be one of the many factors differentiating host populations, thus they may contribute to host reproductive isolation by accelerating or strengthening it (contributor role). To disentangle whether parasites drive or contribute to host divergence, I also analysed infection patterns in replicates of host species pairs (blue and red forms of *Pundamilia* spp.) varying in the age of speciation and extent of genetic differentiation (**chapter 3**). In sympatric and allopatric pairs of *Pundamilia*, divergence in infection profiles increased with host genetic differentiation. This positive relationship was stronger among allopatric pairs than within sympatric pairs, suggesting that parasites may contribute to host divergence in allopatry rather than in sympatry, or alternatively that the time since geographical isolation contributes to both parasite dissimilarity and genetic distance. Within sympatric pairs, infection differences were significant for the old and most genetically differentiated pair (blue and red forms at Makobe), but not for host pairs with low genetic differentiation (except 2 cases). These findings suggest that infection differences only accumulate after host genetic differentiation: a certain amount of genetic differentiation (driven by other factors) may be needed for parasite-mediated divergent selection to act and to lead to significant species differences in infection. This supports a contributor role of parasites in host divergence rather than a driver role, consistent with observations in Lake Tanganyika. There, differences in infection were detected between allopatric populations of *Tropheus moorii* at early stages of diversification (although 15-75'000 years older than populations of *Pundamilia*; Koblmüller et al., 2011), but these did not increase with host neutral genetic differentiation (Hablützel et al., 2016). This supports the hypothesis of a threshold in (neutral) genetic differentiation required for parasite-mediated selection to act.

Species differences in infection can result from host ecology (thereby exposure to parasites), host immune response and interactions between them (Wolinska & King, 2009). In the context of parasite-mediated diversification, species differences in infection profiles are assumed to be initiated by variation in ecological exposure and then maintained by differences in immunological traits. In **chapters 2 and 3**, I found that host ecology (i.e. water depth, diet) played a role in infection heterogeneity but did not fully explain it. Host species identity was the best predictor of infection levels, suggesting that intrinsic factors (e.g. resistance, tolerance) are more important than extrinsic factors of infection variation. For one of the young and weakly differentiated *Pundamilia* pairs (at Python Island), I found that infection differences as in the wild are not maintained in uniform parasite exposure conditions (**chapter 4**). This suggests that the contribution of parasite exposure to infection variation is larger than that of species immunity in recently diverged host species, and it may initiate the species differentiation in infection in the wild. This constitutes no evidence for a contribution of parasites to divergence in *Pundamilia*. However, this does not mean that parasites are not an important dimension of ecological species differentiation: the genetic differentiation between these incipient species is very low and thus

it is not surprising that they may not have diverged in resistance or tolerance yet. Results from similar pairs at more advanced stages of speciation (i.e. *Pundamilia* at Makobe Island) suggest that divergence in resistance/tolerance may become evident as infection differences accumulate (**chapter 3**).

If parasites contribute to the reproductive isolation of *Pundamilia*, then hybrids were expected to be disadvantaged, as a hybrid disadvantage would contribute to reproductive isolation between parental populations. This was not observed in young and closely related *Pundamilia* species from Python Island: first-generation laboratory-bred hybrids did not differ in infection profiles from either parental species (**chapter 4**). Even though hybrids did not have an intrinsic disadvantage in the laboratory, they are rare in the field, likely because of species-assortative mating rather than because of fitness reduction (van der Sluijs et al., 2008a; van der Sluijs et al., 2008b). This suggests that parasites do not contribute to the rarity of hybrids that is observed in the wild and hence additional (ecological) factors may drive assortative mating. This implies that parasites do not drive or strengthen reproductive isolation in *Pundamilia*.

Geographically consistent species differences in infection profiles – Exposure to parasites may depend not only on host ecology, but also on the geographical location where the host occurs. Indeed, chances of getting infected and the number of parasites infecting conspecific host populations varied between locations **(chapters 2 and 3**). For example, the prevalence and abundance of copepods were higher at Makobe than at Kissenda and Python islands (**chapter 3**). However, geographical variation did not seem to affect differences in infection patterns within sympatric host forms. For example, the direction of the infection difference of copepod between blue and red forms was maintained at all sampled locations: red forms of *Pundamilia* tended to harbour more copepods than the blue forms (although this difference was significant only at one location, Makobe). A similar pattern was observed in two Tropheini species co-occurring at several locations in Lake Tanganyika: the direction of species differences in monogenean infection abundance and intensity was maintained despite geographical variation in infection (Grégoir et al., 2015). In addition, differences in parasite community composition were not associated with increasing geographic distance among allopatric pairs of *Pundamilia* (**chapter 3**). The maintenance of the direction of differences in infection patterns within sympatric blue-red forms despite geographical variation in infection suggests that those differences arise because of species-specific host traits (e.g. resistance, host ecology), consistent with parasite-mediated diversification.

7.2. THE ROLE OF *CICHLIDOGYRUS* IN HOST DIVERSIFICATION

The gill parasite *Cichlidogyrus* was the best candidate for testing parasite-mediated speciation, because: *i) Cichlidogyrus* is a species-rich genus, with high morphological diversity, *ii)* species often display high host specificity, infecting only one or few cichlid species, *iii)* it has radiated in at least one other African lake, where it co-evolved with cichlids (Vanhove et al., 2015). To investigate its potential role in Lake Victoria cichlid diversification, I morphologically identified species of *Cichlidogyrus* (described in **chapter 6**). I analysed infection patterns of *Cichlidogyrus* at the level of the species community in each host (hereafter reffered to as *Cichlidogyurs* species level). Results at *Cichlidogyrus* species level are compared to those of higher taxonomic groups (e.g. *Lamproglena*, nematodes), as they often differ.

Divergence in infection of *Cichlidogyrus* between ancient cichlid genera but not between species of the radiation – The first prerequisite for *Cichlidogyrus*-mediated diversification – namely host species differences in infection – was partially met. Similarly to what I observed at parasite higher taxon level, the abundance and community composition of species of *Cichlidogyrus* differed between two sympatric and reproductively isolated species of *Pundamilia* (at Makobe Island, **chapter 3**). However, when considering additional sympatric cichlid species of the adaptive radiation occurring at the same location previously considered (Makobe Island), the community composition of *Cichlidogyrus* species did not differ between cichlid species of the radiation, contrary to the species differences in infection observed at parasite higher taxon level (**chapter 2**). This homogenous infection pattern within the cichlid radiation does not support a role of *Cichlidogyrus* in host diversification, as recently diverged radiation members were expected to evolve species-specific resistance leading to infection divergence. Differentiation in *Cichlidogyrus* infection may take longer time and be visible only between strongly genetically differentiated host species. This hypothesis is supported by two observations. First, *Cichlidogyrus* infection differs between cichlid genera – that diverged more than 5 million years ago – but not between species – that diverged in the past 15'000 years (**chapter 2**). Second, *Cichlidogyrus* infection differs between host species of the nearly 3-4 million years older Tropheini of Lake Tanganyika (Vanhove et al., 2015).

Instead of within-radiation differences, the community composition of *Cichlidogyrus* differed between the three ancient host lineages – the radiating lineage and the two older lineages represented by *Astatoreochromis alluaudi* and *Pseudocrenilabrus multicolor* – indicating a deeper phylogenetic signature. This infection pattern of within-radiation homogeneity and between-lineages differences may result from two alternative scenarios (see **7.3** for further discussion). In the first scenario, *Cichlidogyrus* species sorted among host species during the radiation. In the second scenario, *Cichlidogyrus* species specialised on the radiation lineage as a whole (i.e. all newly evolved cichlid species represent one resource) and subsequently evolved

in the lake. A third scenario, namely ancestral *Cichlidogyrus* species co-evolved with each radiation member, can be excluded because it would result in radiation members differing in infection (which I do not observe). Despite full sympatry of the cichlid hosts investigated, species of *Cichlidogyrus* infecting one lineage rarely infected another lineage, suggesting an opportunity for host specialisation. However, this cannot be linked to parasite-mediated speciation, as the three lineages considered are too distantly related to be informative in such context.

Just like at parasite higher taxon level, I found variation in parasite microhabitat distribution over gills for the two most common species of *Cichlidogyrus*, within and between host species (**chapter 5**). This supports the species-specificity of the cichlid-*Cichlidogyrus* relationship and seems to contrast with the uniform infection pattern found within the radiation. This highlights the importance of exploring more axes of variation in infection (i.e. spatial niche) as it may reveal more differences than canonical measures (i.e. parasite counts).

No contribution of *Cichlidogyrus* to species divergence in *Pundamilia* – As I did for parasites at higher taxon level, I analysed infection patterns in replicate speciation events of blue-red forms of *Pundamilia* to investigate at what stage of host speciation *Cichlidogyrus* species contributed to host divergence (**chapter 3**). Contrary to findings at parasite higher taxon level, infection differences in the community of *Cichlidogyrus* species did not increase with host genetic differentiation within sympatric and allopatric pairs of *Pundamilia*. Among sympatric blue-red forms, only the oldest and most genetically differentiated pair (at Makobe) differed in infection parameters of *Cichlidogyrus* (as found at parasite higher taxon level). A lack of correlation between differentiation in *Cichlidogyrus* infection and host genetic differentiation was also observed at early stages of diversification in Lake Tanganyika (i.e. between allopatric populations of the same cichlid species), although older than *Pundamilia* populations (Grégoir et al., 2015). This indicates that *Cichlidogyrus* infection diverges only when host species have already strongly diverged genetically, whereas at earlier stages of speciation *Cichlidogyrus*-mediated selection does not differ between *Pundamilia* forms. This suggests that species of *Cichlidogyrus* do not contribute to host divergence.

Young blue-red pairs with nearly zero genetic differentiation (at Luanso) and with intermediate levels of genetic differentiation (at Kissenda and Python) did not significantly differ in parasite community composition. Only when host populations reach a certain threshold of genetic differentiation (driven by other factors, mainly ecology-related) they start to diverge in parasite infection. Thus, the extent of differences in infection patterns, thereby their contribution to host divergence, may depend on both host genetic differentiation and ecological divergence.

Geographically consistent species differences in *Cichlidogyrus* infection profiles – Similar to what I observed at parasite higher taxon level, geographical locations differed in the abundance of some species of *Cichlidogyrus* (**chapters 2 and 3**). For example, one host species (*A. alluaudi*)

had higher numbers of *Cichlidogyrus longipenis* at Makobe than at Sweya (**chapter 2**). However, this did not generate differences in the community composition of *Cichlidogyrus* between allopatric host populations of *A. alluaudi*, as proportions between the different species of *Cichlidogyrus* infecting them did not change. In addition, variation in community composition of *Cichlidogyrus* was not associated with geographic distance (**chapter 3**). A similar pattern was observed in allopatric populations of two Tanganyikan cichlids, a strong and a weak disperser (Grégoir et al., 2015). Since infection differentiation between host populations was stronger in the weak disperser than in the strong disperser species, Grégoir et al. (2015) suggested that low host dispersal enhances infection differentiation. In *Pundamilia*, I found indications for the opposite pattern: blue forms, which show isolation by distance (Seehausen et al., 2008; Meier et al., 2017b), have lower inter-population infection differences than red forms. This suggests that infection profiles are species-specific, rather than simply determined by geographic variation in exposure or by connectivity among host populations, in line with parasite-mediated diversification.

7.3. DIRECTIONS FOR FUTURE RESEARCH

Based on the findings presented in this thesis, I propose several directions for future research.

Other adaptive radiations – In this thesis, I investigated the adaptive radiation of haplochromines in Lake Victoria, which is 14'600 years old (Johnson et al., 2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a) and two older lineages, which are 10 million years old (Meier et al., 2017a; Schedel et al., 2019). Many other examples of adaptive radiations exist and could be investigated for parasite-mediated speciation. However, old radiations would be not informative in this context, as it is not possible to discriminate between species differences that arose at the onset of the divergence – possibly driving it – and the many others that accumulated *after* speciation. As presented in **chapters 2 and 3**, the time elapsed since divergence is an important factor determining infection divergence and its detectability. Detectability of infection differences depended also on the taxonomic resolution of parasite identification. At parasite higher taxon level, major infection differences are observed already between species of the radiation (i.e. not later than 14'600 years ago) with low levels of host genetic differentiation (i.e. low F_{ST} values), consistently with a role of parasites in host diversification. On the other hand, at *Cichlidogyrus* species level, infection differences are not observed within the radiation, inconsistently with parasite-mediated speciation (**chapter 2**). Since only young (about 14'600 years old in Victorian cichlids) and old (3-5 million years old in Victorian cichlids, **chapter 2**; and 10 million years in Tanganyikan cichlids, Vanhove et al., 2015) cichlid species were investigated for their divergence in infection, we need to study infection patterns of species with an estimated divergence time that would fill this temporal gap. Since

infection differentiation takes place at different time scales, we need to study infection patterns along a wide range of host divergence time (e.g. from incipient species to species younger than one million years) in order to estimate at what speciation stage infection starts to diverge.

Additional non-speciating lineages – Comparison of infection profiles of *Cichlidogyrus* species between cichlid species revealed a deep phylogenetic split between ancient host genera (**chapter 2**). Three ancient cichlid lineages were considered here: the species-rich radiation lineage and two single-species lineages that never speciated in the Lake Victoria region. Although the latter category is under-represented by definition in terms of species, in Lake Victoria two other species failed to radiate: *Oreochromis variabilis* and *O. esculentus*. Parasitological analysis of these may support the observed pattern if they harbour a set of species of *Cichlidogyrus* that differ from the other lineages. However, both species of *Oreochromis* are critically endangered and it would not be justified to sacrifice individuals solely for this purpose. Individuals of *Oreochromis* kept and bred in cages within the lake may be an option to study their natural infection without impacting on the wild population.

Other species pairs – Beside *Pundamilia*, other species pairs have replicates that vary in the extent of genetic differentiation, ranging from ongoing to recently completed speciation. These offer the opportunity to investigate whether parasites drive or contribute to host differentiation. Examples of speciation replicates from the animal kingdom are: normal-size benthic and dwarf limnetic forms of whitefish (*Coregous clupeaformis*) and wing morphotypes of the *Heliconius* butterflies. Among cichlids, suitable species pairs could be the species of *Neochromis* from Lake Victoria (Magalhaes et al., 2012) and gold/dark colour forms of Midas *Amphilophus citrinellus* from Nicaraguan crater lakes (Kusche et al., 2015). In particular, I find species pairs of *Neochromis* the most promising ones. The genetic differentiation range of *Neochromis* pairs is slightly lower than that of *Pundamilia* pairs (F_{ST} 0.001-0.019 vs. 0.003-0.101), but *Neochromis* are more differentiated in their ecology (especially trophic morphology) than *Pundamilia* (Magalhaes et al., 2012; van Rijssel et al., 2018a). Speciation of *Neochromis* was proposed to be mainly driven by ecological divergence (van Rijssel et al., 2018a). Since ecological differences may be associated with different parasite threats (**chapter 2**; Hablützel et al., 2017), *Neochromis* are expected to respond to parasite-mediated selection.

More precise ecological factors – Species-specific depth distributions and diet play a role in species variation in infection, although these effects depended on the extent of genetic differentiation and age of the host species considered (i.e. outweighed by other intrinsic species traits in reproductively isolated sympatric hosts, but not in younger ones; **chapters 2, 3 and 4**). However, in this thesis I considered only two species-specific ecological traits: water depth and host diet. Since the infection differentiation could depend on every factor that differentiates a host species, it may be worthy to include more and increasingly more specific ecological traits (e.g. in **chapter 2** an increase in resolution of depth categorization lead to larger differences).

Trophic guilds could be split into narrower categories of foraging behaviour (e.g. snail crusher and snail sheller molluscivores) and/or items eaten (e.g. ostracods or bivalves for molluscivores, fly larvae or caddis-fly larvae for insectivores). Feeding ecology could also be assessed through analysis of stable isotope ratios (Muschick et al., 2012). Additional parameters that may explain interspecific variation in infection could include (but are not limited to): host habitat type and spatial range, host population size and density, parasite population size and density.

Assessment of parasite ecological data – In previous studies, ecological data reported concerning parasites are mainly limited to host species. In **chapter 5**, I showed that microhabitat distribution of parasites over gills may represent an important axis of divergence in infection between host species, whereas the parasite reproductive activity is not. Therefore, I recommend to integrate the location of parasites on the host in future studies. Other ecological aspects of parasites that may be relevant for infection variation and host specificity that may be considered in future studies are: aggregation of conspecific parasites, adult body size, rate of infection success.

Host detection by parasites – Pathways of transmission are important in understanding infection patterns. Most ectoparasites studied here actively search for a suitable host in the water column. To recognize a suitable host, parasites are assumed to exploit specific host signals, such as visual or chemical cues. Aquatic parasites with low visual sensitivity are likely to use chemical cues (e.g. substances in skin or gill mucus) to locate their host (Whittington, 1997). Identification of these substances may help to explain differences in parasite abundances observed between host species and differences in infection profiles of *Cichlidogyrus* observed between cichlid lineages. For example, all radiation members may secrete the same set of chemicals (supporting the hypothesis that they are all perceived as one suitable host by the *Cichlidogyrus* species infecting radiation members) but in different quantities (explaining variation in infection levels). Chemicals secreted by radiation members might differ from those of the two non-radiating lineages, explaining the *Cichlidogyrus* infection differences between host lineages. If such chemical cues are species-specific (i.e. attractive only for the *Cichlidogyrus* species actually infecting these hosts), this would constitute support for host-parasite coevolution.

Phylogenetics of *Cichlidogyrus* – The *Cichlidogyrus* community was shared within the Lake Victoria cichlid radiation, but it differed between the radiation and two distantly related cichlid species that did not radiate in the lake (*Astatoreochromis alluaudi* and *Pseudocrenilabrus multicolor*; **chapter 2**). This pattern may result from different mechanisms. It is necessary to perform genetic analysis to date the origin of the species of *Cichlidogyrus* relative to the origin of the Lake Victoria cichlids, in order to understand whether *Cichlidogyrus* species were introduced into the lake with the ancestors of the cichlid radiation (14’600 years ago; Seehausen et al., 2003; Meier et al., 2017a) and then *i)* sorted among cichlid lineages, or *ii)* diversified between cichlid lineages. Since the ancestors of the Lake Victoria cichlid radiation are more

closely related to each other than they are to *A. alluaudi* and *Ps. multicolor*, both scenarios predict that species of *Cichlidogyrus* infecting radiation members are more closely related to each other than those infecting *A. alluaudi* and *Ps. multicolor*. The first scenario also predicts that radiation-infecting species of *Cichlidogyrus* are as young as or younger than the hybrid swarm that gave rise to the cichlid radiation itself (i.e. 14'600 years old or younger). The second scenario predicts that radiation-infecting species of *Cichlidogyrus* are older than the cichlid radiation but younger than the Lake Victoria cichlid superflock (i.e. between 14'600 and 100'000 years old). Alternatively, *Cichlidogyrus* species may have been introduced into the region by the founders of the Lake Victoria Region Superflock (LVRS, about 100'000 years ago; Verheyen et al., 2003; Seehausen, 2006) and then *iii)* sorted over the emerging cichlid species, or *iv)* evolved with the cichlids. The third scenario implies that species of *Cichlidogyrus* are as young as or younger than the origin of the LVRS but older than the radiation (i.e. between 14'600 and 100'000 years old), whereas the fourth scenario predicts that *Cichlidogyrus* species are older than the LVRS (i.e. older than 100'000 years). In addition, genetic analyses may disclose the presence of more *Cichlidogyrus* haplotypes/species than that currently identified with morphological methods, which may have higher host-specificity (both previously observed in monogeneans by Pouyaud et al., 2006 and in trematodes by Jousson et al., 2000; Donald et al., 2004). If such cryptic species are sorted among host species, this would imply that radiation members actually differ in infection profiles of *Cichlidogyrus*, possibly supporting parasite-mediated speciation.

Extrinsic vs. intrinsic traits – My findings suggest that variation in parasite exposure contributed to variation in infection among host species (**chapters 2, 3 and 4**), while host immunity-related intrinsic traits did not (at least between young host species; **chapter 4**). Anyway, I cannot exclude that older host species may have intrinsic differences (i.e. host immunity). This can be assessed by comparing species differences in infection profiles between wild and laboratory-bred fish of species pairs with low, intermediate and high levels of genetic differentiation (similar to what I did for the incipient species of *Pundamilia* from Python Island). Maintenance of species differences in infection under uniform exposure would indicate that differences in resistance (or other immune-related traits) have evolved. This would support a role of parasites in strengthening host divergence if immunity differences arise before the completion of reproductive isolation. In addition, MHC genotyping can reveal whether species within a pair differ in resistance (see below).

Hybrid disadvantage – In the context of parasite-mediated divergence, hybrids are expected to be more infected than either parental species (Schluter, 2001), contributing to reproductive isolation. In the field, hybrids of blue and red *Pundamilia* are rare, suggesting some selection against them. However, such hybrid disadvantage was not observed in laboratory conditions (**chapter 4**), suggesting that selection against hybrids is not exerted by parasites. Since the frequency of hybrids varies across wild populations of *Pundamilia* (Seehausen et al., 2008), I propose to explore whether the extent of hybridisation in wild host populations (as a measure

of reproductive isolation) is associated with the infection level of hybrids. I expect that the greater the genetic/phenotype differentiation between host species, the lower the fitness in hybrids and consequently the stronger the assortative mating (Stelkens & Seehausen, 2009). Hybrids of reproductively isolated *Pundamilia* (e.g. at Makobe Island) do not occur in the field, but they can be obtained by housing heterospecific together in semi-natural conditions (as done in **chapter 4** for Python populations, in Lake Tanganyika cichlids by Rajkov et al., 2018). However, it is possible that most pairs of *Pundamilia* are too closely related to reveal an association between hybridisation and infection. Differences in infection, resistance and hybrid disadvantage may become evident after a certain threshold of host divergence (as suggested in **chapter 2**). This would be consistent with parasites contributing to – but not driving – host divergence.

Fitness costs of parasite infection – Throughout this thesis, I have assumed that parasites impose a fitness cost on hosts. Although this is widely assumed, experimental evidence quantifying the parasite impact of each species in single- or multiple species infection is still scarce. Future research would need to address whether and how parasites exert selection on cichlids and to what extent infection is costly. Since natural populations often harbour more than one parasite species, it is also important to investigate multi-species infections. Moreover, single-species infections may be not costly in itself, but may increase vulnerability to other infections or may become more/less costly in the presence of another parasite. Infection cost can be estimated by assessing local damage (i.e. histopathological responses at the attachment site; Reda & El-Naggar, 2003; Arafa et al., 2009; Igeh & Avenant-Oldewage, 2020) and indirect costs on life-history traits (Barker et al., 2002; Bollache, 2015). The latter can be estimated by comparing survival, growth, male nuptial coloration and reproductive output between parasite-free fish and conspecifics with infection at increasing intensities. A complementary approach would also address *how* hosts respond to parasitic infection and if this differs between host species. This can be done by analysing blood (e.g. cytokines, serum protein, immunoglobulins), mucus production or even expression of immune-related genes of experimentally infected fish (as recently done in Nile tilapia by Zhi et al., 2018; Chen et al., 2019).

Host divergence in immunity – Parasites could drive genetic adaptations in host immune resistance, such as the Major Histocompatibility Complex (MHC) (Haldane, 1949; Klein et al., 1994). MHC genes can be subject to divergent selection by local parasites (in sticklebacks, Eizaguirre et al., 2012a, b; in Lake Tanganyika cichlids, Hablützel et al., 2016). The gene pool of MHC varies between cichlid species of Lake Malawi (Klein et al., 1993; Ono et al., 1993; Blais et al., 2007), Lake Tanganyika (Hablützel et al., 2013; Hablützel et al., 2016) and of Nicaraguan lakes (Hofmann et al., 2017). On the other hand, a large sharing of MHC alleles and polymorphisms are observed in cichlids of Lake Victoria (Nagl et al., 1998; Klein et al., 2007). Assessing the MHC diversity of our sampled cichlids could reveal whether species differences in resistance alleles are associated with species differences in infection. Immunogenetic differentiation is particularly

relevant in early stages of host divergence: in the context of parasite-mediated divergence, MHC differentiation is expected to precede neutral genetic differentiation. This pattern was observed in allopatric populations of a Tanganyikan cichlid (Hablützel et al., 2016), in sympatric closely related cichlids in Lake Malawi (Blais et al., 2007) and in sympatric limnetic/benthic populations of lake sticklebacks (Matthews et al., 2010).

Individual variation in parasite resistance – Throughout this thesis, I investigated infection patterns at interspecific level. Future work may address infection differences at host individual level to investigate the heritability of parasite defence strategies – which is necessary for parasites to contribute to host divergence. The heritability of parasite resistance has been tested in only few vertebrate organisms (soay sheep Smith *et al.* 1999, kittiwakes Boulinier et al., 1997, barn swallow Moller 1990), including fish (beaked dace cyprind; Mazé-Guilmo et al., 2014) but not in cichlids. To experimentally test the heritability of resistance, researchers should perform intraspecific crosses between individuals with high and low resistance. The offspring resistance is expected to be determined by the parental resistance. If this is the case, researchers could take a step further and investigate whether such heritable variation in resistance is linked to mate selection based on resistance. Host individuals are expected to mate with the most resistant partners, in order to generate a resistant offspring. Such link between female mate choice and parasite load was observed only indirectly in *Pundamilia*: mate choice is based on male red coloration, which is associated with parasite load (Maan et al., 2006b; Maan et al., 2008) and with antibody response (Dijkstra et al., 2007). If different signals become associated with heritable immunity in different subpopulations, then mate choice might be mediated by parasite resistance, which could contribute to parasite-mediated divergence.

7.4. CONCLUSION

In this thesis, I investigated parasite infection patterns of Lake Victoria cichlids, in order to contribute to the understanding of ecological speciation. My findings allow the following general conclusions.

First, parasites are non-randomly distributed across host species, despite their host full sympatry (**chapters 2 and 3**). This is a requirement for a role of parasites in host differentiation (Karvonen & Seehausen, 2012) and is consistent with parasite specialisation. Also within hosts, I observed non-random distributions of parasites: parasites were more frequent in certain gill microhabitats and this niche distribution differed across host species in some cases (**chapter 5**).

Second, species differences in infection profiles were temporally consistent (**chapters 2 and 3**). This indicates that another prerequisite for parasite-mediated selection is met: parasite-mediated divergent selection maintains its direction over time.

Third, when host species start to be genetically differentiated, they also begin to accumulate differences in parasite communities (**chapters 2, 3, 4**). This implies that differentiation in parasite infection arise during the divergence process, but is not driving it. In young host species, that hardly differ genetically, differences in infection as observed in the wild disappear when equalizing exposure (**chapter 4**), indicating that exposure may drive the onset of infection differentiation, rather than defence-related species factors (e.g. immunological traits).

Fourth, infection profiles of old species strongly differ from those of young species. This deep phylogenetic signature in infection is consistent with parasite specialisation, but not with parasite-mediated speciation.

Fifth, patterns observed at parasite higher taxon level differ from those at within-genus level in *i)* infection profiles across hosts, *ii)* gill niche distribution and *iii)* parasite-parasite interspecific interactions within hosts. Infection profiles differed between host species of the radiation when considering parasite genera, whereas they did not when considering species of *Cichlidogyrus*. Niches in the gills differed between parasite genera, whereas niches overlapped among species of *Cichlidogyrus*. Interspecific interactions were synergistic among parasite genera, whereas they were antagonistic among species of *Cichlidogyrus*. This may be explained by the higher similarity at within-genus level than between higher taxon level.

Based on previous studies, I considered *Cichlidogyrus* the main candidate for driving parasite-mediated speciation. In this thesis, I did not observe such pattern, as infection differences of *Cichlidogyrus* only become evident between distantly related species, after differences in infection of other parasite taxa. This may have three explanations. First, the fitness cost of *Cichlidogyrus* may be too low at natural infection levels to exert selection for specialised resistance in the host, inconsistent with parasite-mediated diversification. Alternatively, the imposed fitness cost may be so high to kill highly infected hosts before they can be sampled, hampering any conclusion from field studies. Second, although a young radiation is a good model to study mechanisms of host speciation, the cichlid radiation in Lake Victoria may be too young to allow detection of patterns of cichlid speciation mediated by monogeneans. Third, the diversity of *Cichlidogyrus* may have been underestimated by morphological identification. Molecular investigations may actually reveal an infection pattern that is consistent with parasite-mediated diversification. Since most observed species of *Cichlidogyrus* were new to science and genetic data are currently lacking, I cannot estimate the age of these species. However, I can speculate that these species of *Cichlidogyrus* are endemic to the Lake Victoria basin (or even to the lake itself) (**chapter 6**). The potential endemism of *Cichlidogyrus* species of Lake Victoria suggests that these species may have evolved in the basin or even in the lake (contrary to the globally distributed copepod species observed there). Thus, comparing infection patterns of Victoria cichlids with *Cichlidogyrus* species and those with copepod species can help us to distinguish recent from ancient eco-evolutionary mechanisms in the host-parasite interactions.

Although most evidence supporting parasite-mediated speciation currently comes from sticklebacks (e.g. Milinski & Bakker, 1990; Wegner et al., 2003; Eizaguirre et al., 2009a; MacColl, 2009b; Matthews et al., 2010; Eizaguirre et al., 2012a), African cichlids have the potential to diverge in response to parasite, as they harbour a high richness of parasite species (this thesis, Raeymaekers et al., 2013; Vanhove et al., 2015) and they have a diverse MHC that allows them to rapidly adapt to local parasite threats (Blais et al., 2007). Cichlids also have the advantage to provide many cases of young closely related species along with species that never diversified, allowing the study of early stages of host divergence and comparison with "diversification failures".

Although the evidence for a role of parasites in driving or contributing to host differentiation is increasing (this thesis; Eizaguirre et al., 2009a; Raeymaekers et al., 2013), we are still scratching the surface of the potential evolutionary impact of parasites on hosts and further research is much needed. African cichlids are a promising model system in the context of parasite-mediated speciation but also for other researches linked to parasites. For example, the parasite fauna of Lakes Victoria and Malawi is largely unknown and even in Lake Tanganyika (the most investigated of the three Great Lakes for parasitology) studies on parasite ecology, life history and genomics are needed. Additional future research may also focus on factors determining the host-parasite interaction, on host (divergent) adaptations in response to (different) infections, on the interplay of parasites and other (ecological) factors contributing to host divergence, and on mechanisms of host reproductive isolation driven by parasite-mediated selection (especially those mediated by immune traits).

8

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English summary

Tiziana P Gobbin

PARASITE-MEDIATED SPECIATION

Speciation – the formation of new species – was defined by Darwin as the "mystery of mysteries" more than a century ago. Since then, mechanisms of speciation have been investigated intensively and progress has been made. Adaptations to biotic and abiotic factors may cause speciation as a by-product, and parasites may be an important biotic agent of selection. However, some mechanisms remain under-explored. In particular, the onset of divergence is still not well understood. In this thesis, I investigate when and how parasite-mediated divergent selection contributes to speciation process.

Parasites constitute a widespread source of ecological selection (Poulin & Morand, 2000; Schmid-Hempel, 2013), that may potentially act as a driver of speciation (Schluter, 1996, 2000b; Rundle & Nosil, 2005; Maan & Seehausen, 2011). By definition, parasites impose fitness costs on their hosts (e.g. reduced growth, reproduction and survival, Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018). Hosts adapt to parasites by evolving resistance, tolerance or behavioural avoidance. In turn, parasites counter-adapt by evading or suppressing host immunity. This leads to a coevolutionary dynamic of adaptation and counter-adaptation (Decaestecker et al., 2007).

Host populations occupying different ecological niches may be exposed to different parasite numbers and species, potentially resulting in different parasite-mediated selection (Knudsen et al., 2004; Pegg et al., 2015; Hablützel et al., 2017; Hayward et al., 2017) even in sympatry. This may lead host populations to evolve different adaptations against local parasite threats. Such adaptive responses can lead to an increasingly different parasite infection pattern between host populations. If these differences are maintained over time, then parasite-mediated selection continuously acts in the same direction, promoting host divergence. Stochastic or frequency-dependent temporal fluctuations in parasite abundances could cause variation in the strength of parasite-mediated selection, but divergence is promoted as long as the direction of selection is maintained (**Fig. 9.1**).

Such divergent and temporally stable differences in infection may lead to genetic differentiation between host populations, and eventually drive or strengthen reproductive isolation between them (Hamilton & Zuk, 1982; Landry et al., 2001; Nosil et al., 2005; Maan et al., 2008; Eizaguirre et al., 2011). Moreover, reproductive isolation could be reinforced by selection against hybrids and immigrants (i.e. with higher infection), immune-mediated mate choice (i.e. choice for partners providing locally adaptive immunity) or parasite-mediated mate choice (i.e. choice for healthy partners). Alternatively, parasite-mediated divergent selection may strengthen host differentiation once a certain level of reproductive isolation is already achieved through other mechanisms (Haldane, 1949; Price et al., 1986; Karvonen & Seehausen, 2012).

The study of parasite-mediated speciation can be problematic because of the two-way nature of the host-parasite interaction – one needs to determine which of the two is driving the diversification – and because of the involvement of other ecological factors – one needs to distinguish the effects of parasites from those of other potential drivers of diversification. To this end, host populations at early stages of speciation provide a good model system. In this thesis, I take advantage of the young adaptive radiation of cichlid fish in Lake Victoria to investigate the role of parasites in host speciation.

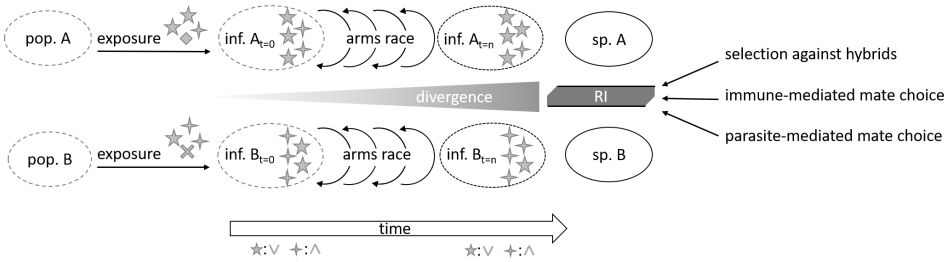


Figure 9.1
Parasite-mediated speciation. Two interbreeding host populations occupy two different ecological niches (A and B) and are exposed to different numbers and species of parasites (symbols), resulting in two different infection patterns. Each host population evolves adaptations against local parasites, engaging in an evolutionary arms race. The direction of infection differences remains consistent over time despite fluctuations in overall abundance (★ higher in population A than in B, ✦ lower in A than in B). Divergence in defences against parasites leads to reproductive isolation (RI) between host populations – that may be reinforced by selection against hybrids and/or immigrants, and/or immune-mediated and/or parasite-mediated mate choice– resulting in two distinct host species.

LAKE VICTORIA CICHLIDS AND THEIR PARASITES

The adaptive radiation of cichlid fish in Lake Victoria is particularly suitable for studying parasite-mediated speciation, because of its young age, interspecific ecological diversity and relatively weak genetic differentiation. As recently as 14'600 years ago, the lake refilled after being dry for thousands of years (Johnson et al., 1996; Stager & Johnson, 2008). A hybrid swarm formed after colonization of the refilled lake by two riverine lineages (Seehausen et al., 2003; Meier et al., 2017a), providing the genetic variation that facilitated the rapid adaptive speciation (Seehausen, 2004; Salzburger, 2018). Thus, most Victorian cichlids evolved *in situ* after that dry period (Johnson et al., 2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a). In Lake Victoria, radiation members co-occur with old and distantly related lineages that did not speciate after colonizing the lake: *Astatoreochromis alluaudi* (Pellegrin, 1904), *Pseudocrenilabrus*

multicolor (Schöller, 1903), *Oreochromis variabilis* (Boulenger, 1906) and *Oreochromis esculentus* (Graham, 1928). These provide a helpful comparison to study why some lineages speciated and others did not.

Previous studies suggest that parasite-mediated speciation might happen in cichlids, because of strong diversity in ecological niches (Fryer & Iles, 1972; Wagner et al., 2012a), high potential for disease transmission because of high fish densities (Ribbink et al., 1983; Fenton et al., 2002), association between parasitism and the expression of sexual signals (Taylor et al., 1998; Maan et al., 2006b) and rapid evolution of MHC genes (Blais et al., 2007). Moreover, there is evidence for co-evolution between cichlids of Lake Tanganyika and their monogenean gill parasites (Vanhove et al., 2015).

Lake Victoria also harbours replicates of speciation at different stages, which allows to assess when – during the speciation process – differences in infection arise. The blue *Pundamilia pundamilia* (Seehausen et al., 1998) and the red *Pundamilia nyererei* (Witte-Maas and Witte, 1985) are two closely related cichlids that co-occur at rocky islands in the southeastern part of the lake. At some locations, these two species hybridized and then speciated again into similar blue and red pairs (Meier et al., 2017b; Meier et al., 2018). Across locations, blue and red forms vary in the extent of genetic differentiation (Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018), morphological differentiation (van Rijssel et al., 2018a), differentiation in visual adaptation (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019), and in the frequency of hybridisation (Seehausen, 1996a; Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018). Variation in these traits is associated with water transparency.

Parasites live at the expenses of their hosts, thereby imposing a fitness cost. The parasite life cycle can entail one or more host species (the final host harbouring reproductive adults) and may also include free-living stages (often eggs or larvae). Cichlids are hosts to many macroparasite taxa: monogeneans (gill parasites, but some genera infect digestive tract or bladder, with a direct life cycle), copepods (gill or skin parasites, with a direct life cycle), bivalve molluscs (gill or skin parasites, with a direct life cycle), nematodes (endoparasites, often fish are intermediate hosts), trematodes (flukes, endoparasites with at least two intermediate hosts). Monogeneans are of great interest in the study of host-parasite interactions because of their high host specificity. In particular, the gill parasite *Cichlidogyrus* is a good candidate for promoting host speciation, because of its high number of species, that differ in morphology, they display a high host specificity (Pariselle et al., 2003; Vanhove & Huyse, 2015; Vanhove et al., 2015).

THIS THESIS

In this thesis, I investigate whether parasites drive or contribute to host speciation in cichlid fish of Lake Victoria. To this end, I analysed the macroparasite infection of a large sympatric community of 17 cichlid species that radiated and two species representing lineages that never speciated (**chapters 2 and 5**), as well as infection differences between four replicates of blue and red *Pundamilia* pairs that vary in the extent of genetic differentiation (**chapters 3 and 4**). Fish were infected by five genera of gill parasites (*Cichlidogyrus* spp., *Gyrodactylus sturmbaueri*, *Lamproglena monodi*, *Ergasilus lamellifer*, glochidia larvae of bivalves) and two endoparasites in the abdominal cavity (nematodes, trematodes). Despite being good candidate for promoting cichlid speciation, *Cichlidogyrus* of Lake Victoria are mostly unknown. Thus, I morphologically identified them to species level. Infection patterns were analysed at between parasite genera level for two sampling years (2010 and 2014) and at within-*Cichlidogyrus* level for one sampling year (2014). Since results differed according to the parasite level analysed, I present them separately.

Infection differences at parasite higher taxon level

In **chapters 2 and 3** I tested two prerequisites for parasite-mediated speciation: *i)* species differences in infection and *ii)* temporal consistency in the direction of parasite-mediated selection (Karvonen & Seehausen, 2012). Seventeen sympatric host species occurring at Makobe and four pairs of blue and red forms of *Pundamilia* at four locations differed in their parasite infection, both in terms of parasite abundance and diversity, consistent with divergent parasite-mediated selection. These infection differences were mostly consistent between the two sampling years within all sampled species of the radiation and within the reproductively isolated sister species, supporting the temporal consistency hypothesis for parasite-mediated speciation.

In **chapter 3**, I assessed whether infection differences between species of blue and red *Pundamilia*, from four locations, covary with the extent of genetic or geographic distance between them. Only the most genetically differentiated blue-red sympatric pair differed in infection profiles. Comparison of all species pairs (sympatric and allopatric, of same and different colour) revealed that the extent of parasite community dissimilarity increased with increasing genetic distance within pairs, taking into account geographic distance among islands. These results suggest that species differences in infection depend on the extent of host genetic differentiation: infection differences accumulate as host genetic divergence increases, rather than precede genetic divergence. Therefore, parasites may contribute to host species differentiation but do not drive it. The positive correlation between infection differentiation and genetic differentiation at parasite higher taxon level was observed in both sampling years, supporting the consistency of parasite-mediated selection over time.

Although the intensity of some parasites was associated with water depth, variation in infection among host species was not fully explained by water depth and trophic specialization (**chapters 2 and 3**). This suggests that other intrinsic species properties (i.e. immunity and genetic susceptibility) also play a role. In **chapter 4**, I investigated the contribution of host intrinsic properties to variation in infection, by assessing infection differences in laboratory conditions with uniform exposure. I compared infection patterns between wild caught and first-generation lab-reared hosts of one of the *Pundamilia* pairs of chapter 3, as well as lab-reared interspecific hybrids. Prevalence and abundance of three of the most common ectoparasites were similar between lab and field. The two species differed in infection in the wild but not in laboratory conditions, where fish cannot express some species-specific ecological traits (e.g. depth and diet preferences). This indicates that variation in infection is mainly due to extrinsic effects rather than genetically based species differences in immunity. Since this pair of *Pundamilia* is weakly genetically differentiated, it is unlikely that differences in immune traits evolved already at early stages of speciation, which is inconsistent with a parasite contribution to divergence of *Pundamilia*.

Hybrids did not differ in infection from either parental species (all lab-bred first-generation, **chapter 4**), inconsistent with a hybrid disadvantage that would promote parasite-mediated diversification. Despite this, hybrids are rare in the field, likely because of species-assortative mating. The lack of hybrid disadvantage suggests that assortative mating is driven by other ecological factors.

In the wild, because of depth segregation, the two species of *Pundamilia* are adapted to different visual environments: blue forms inhabit a broad-spectrum light environment, while red forms inhabit a red-shifted light environment. These two visual conditions were mimicked in the laboratory. A mismatch in the visual environment of the hosts coincides with lower survival (Maan et al., 2017) and may coincide with higher parasite infection. This was not observed: parasite infection did not differ between natural and unnatural light conditions, suggesting that a visual mismatch does not increase host susceptibility.

Infection differences at within-*Cichlidogyrus* level

Contrary to what found at parasite higher taxon level, the *Cichlidogyrus* species community composition was similar within the sampled species belonging to the Lake Victoria radiation (**chapter 2**). This does not support a role of *Cichlidogyrus* in host diversification, as recently diverged radiation members were expected to evolve species-specific resistance linked to infection divergence. Instead, community composition of species of *Cichlidogyrus* differed between the three host lineages – the radiation lineage and the two older lineages represented by *A. alluaudi* and *Ps. multicolor*. Despite full sympatry of the hosts, *Cichlidogyrus* species infecting one lineage rarely infected another lineage, suggesting an opportunity for host

specialisation (although radiation members currently do not represent different resources for species of *Cichlidogyrus*).

When focusing on all pairs of *Pundamilia*, there was no gradual increase in the extent of dissimilarity in the community of *Cichlidogyrus* with genetic differentiation between populations (**chapter 3**), contrary to what found at parasite higher taxon level. As observed at parasite higher taxon level, the only sympatric comparison where community composition of *Cichlidogyrus* differed was in the reproductively isolated blue-red pair at Makobe. This indicates that *Cichlidogyrus* is not driving differentiation in *Pundamilia*. Instead, this suggests that differences in infection arise when hosts have already achieved a certain extent of divergence, contrary to a role of *Cichlidogyrus* in the early stages of host diversification. Together, these results suggests that species of *Cichlidogyrus* do not contribute to host differentiation.

No geographical pattern in species infection differences

Chances of getting infected and the numbers of parasite infecting hosts varied between locations. This may be explained by ecological differences between locations. For example, the highest infection levels of nematodes (often transmitted by birds) were observed at Makobe island, where large populations of cormorants and egrets occur. Abundances of parasites were generally low at the swampy location with few fish species and individuals, compared to rocky islands with relatively large cichlid populations. Despite such geographical variation in infection levels, species differences in infection were consistent across locations (**chapters 2 and 3**). This pattern was observed for single parasite taxa (e.g. red forms of *Pundamilia* harboured consistently more *L. monodi* and *E. lamellifer* than the blue forms) but also at the parasite community composition level (an increase in geographical distance between populations did not coincide with an increase in parasite community dissimilarity). An absence of geographical pattern in sympatric species differences in infection may support a sympatric parasite-mediated scenario, as differentiation in infection may result from intrinsic host traits (including resistance).

Parasite microhabitat segregation

In **chapter 5**, I analysed the micro-habitat distribution of parasites on the gills, to assess whether this could constitute another axis of divergence in infection. The two most abundant ectoparasite taxa (*Cichlidogyrus* spp., *L. monodi*) and species of *Cichlidogyrus* (*C. nyanza*, *C. furu*) had non-random microhabitat distributions that differed between host species, suggesting that the same parasite may interact differently with different host species. This may provide opportunity for parasite-mediated host differentiation. Microhabitat selection represents another axis of infection heterogeneity that may reveal more differences than parasite counts, hence is worthy including in future studies. Parasite interspecific relationships did not differ between host species. In monogeneans it may be explained by increasing opportunities of

mating (as they reproduce on the host); whereas in copepods it may be explained by egg exposure to water flow (as most copepods are attached in a way that exposes egg clutches outside gill filaments).

Within host parasite dynamics

In **chapter 5**, I observed positive correlations between the abundances of ectoparasite taxa and negative correlations between species of *Cichlidogyrus*. Positive relationships may be explained in two ways: *i)* they are true synergistic interactions, potentially resulting from parasite antigenic similarity that allows exploitation of immunomodulation by the other parasite *ii)* they result from being associated with same host ecological specialisation. Negative relationships may be due to competition, possibly related to parasite phylogenetic relatedness or on similarity in resource requirements. The direction and strength of parasite interactions did not differ between host species, suggesting that intrinsic host species traits do not influence parasite relationships, inconsistent with host specificity.

I also explored differences in reproductive activity of copepods (measured as the proportion of females carrying egg clutches) between host species, and observed no differences, among the wild caught species (**chapter 5**) and among the two lab-reared species of *Pundamilia* and their interspecific hybrids (**chapter 4**). This suggests no host specificity of copepod reproductive activity, although varying in infection prevalence and abundance.

CONCLUSION

In this thesis, I found support for parasites in contributing to host divergence, but not in initiating it. First, parasites are non-randomly distributed at least at three levels – gill microhabitat, host species, host lineages – indicating host specialisation and an opportunity for heterogenous parasite-mediated selection. Second, species differences in infection were temporally consistent, in line with prerequisites for parasite-mediated speciation. Third, when host species start to diverge in ecology, they also begin to accumulate differences in parasite communities, suggesting that differentiation in infection is a by-product of divergence rather than the opposite.

Nederlandse Samenvatting

Translation by Gerrit Potkamp

PARASITE-MEDIATED SPECIATION

Soortvorming – het ontstaan van nieuwe soorten – werd meer dan een eeuw geleden door Darwin gedefinieerd als het “mysterie der mysteries”. Sindsdien zijn de mechanismen van soortvorming intensief onderzocht en is veel voorruitgang geboekt. Aanpassingen aan biotische en abiotische factoren kunnen leiden tot soortvorming als een bijproduct, en parasieten kunnen een belangrijke biotische factor voor selectie zijn. Sommige mechanismen blijven echter onderbelicht in het onderzoek naar soortvorming. Die mechanismen achter het begin van divergentie in het bijzonder zijn nog steeds onduidelijk. In deze thesis onderzoek ik wanneer en hoe door parasieten gefaciliteerde selectie bijdraagt tot het proces van soortvorming.

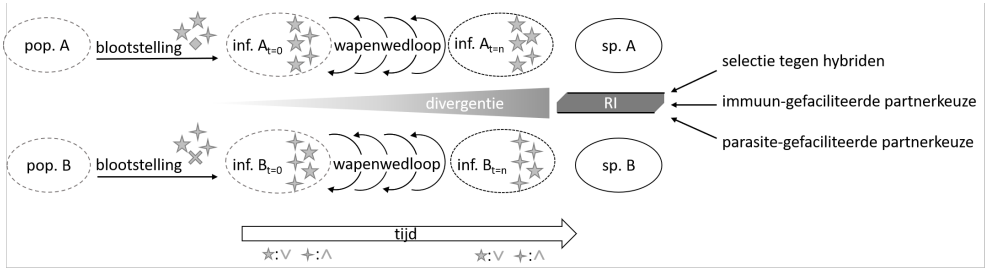
Parasieten vormen een wijdverspreide bron van ecologische selectie (Poulin & Morand, 2000; Schmid-Hempel, 2013), die mogelijk kan functioneren als een aandrijver van soortvorming (Schluter, 1996, 2000b; Rundle & Nosil, 2005; Maan & Seehausen, 2011). Parasieten hebben per definitie een negatief effect op de fitness van hun gastheer (bijvoorbeeld een verminderde groei, reproductie en overleving, Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018). Gastheren passen zich aan parasieten aan door het ontwikkelen van resistentie, tolerantie of vermijding van parasieten door aanpassingen in het gedrag van de gastheer. Dit leidt tot een co-evolutionaire dynamiek van aanpassing en tegenaanpassing (Decaestecker et al., 2007).

Gastheerpopulaties die verschillende ecologische niches bezetten kunnen worden blootgesteld aan verschillende aantallen en soorten parasieten, wat mogelijk kan leiden tot verschillen in door parasieten-gefaciliteerde selectiedruk (Knudsen et al., 2004; Pegg et al., 2015; Hablützel et al., 2017; Hayward et al., 2017), zelfs in sympatrie. Dit kan ervoor zorgen dat gastheerpopulaties verschillende adaptaties ontwikkelen tegen de lokale dreigingen van parasieten. Deze adaptieve reacties kunnen leiden tot een steeds verder differentiërend patroon van infectie door parasieten tussen verschillende gastheerpopulaties. De door parasieten gefaciliteerde selectie treedt doorlopend op in dezelfde richting als deze verschillen over de tijd standhouden, en bevordert op deze manier divergentie tussen gastheren. Stochastische en frequentie-afhankelijke fluctuaties over de tijd in de dichtheden van parasieten kunnen variatie in de sterkte van parasiet- gefaciliteerde selectie veroorzaken, maar divergentie wordt bevorderd zolang de richting van selectie wordt behouden (**Fig. 10.1**).

Verschillen als deze, stabiel over de tijd, kunnen leiden tot genetische differentiatie tussen gastheerpopulaties, en kunnen uiteindelijk reproductieve isolatie tussen populaties stimuleren en versterken (Hamilton & Zuk, 1982; Landry et al., 2001; Nosil et al., 2005; Maan et al., 2008; Eizaguirre et al., 2011). Bovendien, reproductieve isolatie kan worden versterkt door selectie tegen hybriden en immigranten (met een hogere graad van infectie), immuun-gefaciliteerde partnerkeuze (in andere woorden, een keuze voor partners die lokaal adaptieve immuniteit

bieden) of parasiet-gefaciliteerde partnerkeuze (een keuze voor gezonde partners). Als alternatief kan divergente, door parasieten gefaciliteerde selectie gastheerdifferentiatie versterken wanneer een bepaald niveau van reproductieve isolatie is bereikt door andere mechanismen (Haldane, 1949; Price et al., 1986; Karvonen & Seehausen, 2012).

De studie naar parasiet- gefaciliteerde soortvorming kan problematisch zijn vanwege de tweezijdige aard van de interactie tussen gastheer en parasiet – men moet bepalen welke van de twee de diversificatie drijft – en vanwege de betrokkenheid van andere ecologische factoren – men moet de effecten van parasieten onderscheiden van andere mogelijke drijvers van diversificatie. Gastheerpopulaties in een vroeg stadium van soortvorming zijn daarom een goed modelsysteem. In deze thesis maak ik gebruik van de jonge, adaptieve radiatie van cichlide vissen in het Victoriameer om de rol van parasieten in de soortvorming van gastheren te onderzoeken.



Figuur 10.1
Parasiet-gefaciliteerde soortvorming. Twee kruisende gastheerpopulaties bezetten twee verschillende ecologische niches (A en B) en worden blootgesteld aan verschillende aantallen en soorten parasieten (symbolen), wat leidt tot twee verschillende patronen van infectie. Elke gastheerpopulatie ontwikkelt aanpassingen tegen de lokale parasieten, deelnemend aan een wapenwedloop tussen gastheer en parasiet. De richt van verschillen in infectie blijft consistent over de tijd, ondanks verschillen in totale hoeveelheid (★ hoger in populatie A dan in B, ✦ lager in A dan in B). Divergentie in de verdediging tegen parasieten leidt tot reproductieve isolatie (RI) tussen gastheerpopulaties – die kunnen worden versterkt door selectie tegen hybriden en immigranten, en/of immuun-gefaciliteerde en/of parasiet-gefaciliteerde partnerkeuze – resulterend in twee verschillende gastheersoorten.

CICHLIDEN UIT HET VICTORIAMEER EN HUN PARASIETEN

De adaptieve radiatie van cichliden in het Victoriameer is bijzonder geschikt voor het bestuderen van parasiet-gefaciliteerde soortvorming vanwege haar jonge leeftijd, ecologische diversiteit tussen soorten en relatief zwakke genetische differentiatie. Het meer vulde zich slechts 14.600 jaar geleden opnieuw met water na duizenden jaren te hebben droog gestaan (Johnson et al., 1996; Stager & Johnson, 2008). Na kolonisatie van twee lijnes van rivier-cichliden vormde zich een zwerm van hybriden in het meer (Seehausen et al., 2003; Meier et al., 2017a) die de genetische variatie verschafte voor het faciliteren van de snelle adaptieve soortvorming

(Seehausen, 2004; Salzburger, 2018). De meeste Victoria cichliden onstonden dus *in situ* na de droge periode (Johnson et al., 2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a). In het Victoriameer, leden van deze radiatie komen samen voor met oude, ver verwante lijnen die niet *in situ* ontstonden na de kolonisatie van het meer: *Astatoreochromis alluaudi* (Pellegrin, 1904), *Pseudocrenilabrus multicolor* (Schöller, 1903), *Oreochromis variabilis* (Boulenger, 1906) en *Oreochromis esculentus* (Graham, 1928). Deze soorten bieden een nuttig vergelijkingsmateriaal in het bestuderen van de vraag waarom binnen sommige takken nieuwe soorten ontstonden en binnen andere niet.

Voorgaande studies suggereren dat parasiet-gefaciliteerde soortvorming een rol zou kunnen spelen in cichliden, vanwege hun hoge diversiteit in ecologische niches (Fryer & Iles, 1972; Wagner et al., 2012a), een hoge potentie voor overdracht van ziektes vanwege hoge dichtheden van vissen (Ribbink et al., 1983; Fenton et al., 2002), associatie tussen parasitisme en seksuele signalen (Taylor et al., 1998; Maan et al., 2006b) en snelle evolutie in MHC genen (Blais et al., 2007). Er zijn bovendien aanwijzingen voor co-evolutie tussen cichliden uit het Tanganyika-meer en hun monogeneane kieuwparasieten (Vanhove et al., 2015).

Daarnaast herbergt het Victoriameer meerdere voorbeelden van soortvorming in verschillende stadia, waardoor kan worden onderzocht wanneer – in het proces van soortvorming – verschillen in infectie ontstaan. De blauwe *Pundamilia pundamilia* (Seehausen et al., 1998) en de rode *Pundamilia nyererei* (Witte-Maas and Witte, 1985) zijn twee nauwverwante cichliden die samen voorkomen in de rotsige habitats van het zuidoostelijke deel van het meer. Op sommige locaties kruisten deze twee soorten en vormden daarna opnieuw vergelijkbare blauwe en rode varianten (Meier et al., 2017b; Meier et al., 2018). De verschillende blauwe en rode vormen variëren over de verschillende locaties in de mate van genetische differentiatie (Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018), morfologische differentiatie (van Rijssel et al., 2018a), differentiatie in visuele adaptatie (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019), en in de frequentie van hybridisatie (Seehausen, 1996a; Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018). Variatie in deze kenmerken is geassocieerd met de helderheid van het water.

Parasieten leven ten koste van hun gastheren, en daarmee de fitness van hun gastheren. De levenscyclus van een parasiet is afhankelijk van van één of meerdere gastheersoorten (waarbij reproductieve, volwassen parasieten de laatste gastheer infecteren) en kan ook vrijlevende stadia bevatten (vaak eieren of larven). Cichliden zijn gastheer voor vele macro-parasitaire taxa: monogeneanen (kieuwparasieten met een directe levenscyclus, sommige genera infecteren het spijsverteringskanaal of de blaas), copepoden (kieuw- of huidparasitien met een directe levenscyclus), bivalven (kieuw- of huidparasitien met een directe levenscyclus), nematoden (endoparasieten, waarbij vissen vaak een intermediaire gastheer zijn), trematoden (zuigwormen, endoparasieten met tenminste twee intermediaire gastheren). Monogeneanen

zijn van groot belang in het bestuderen van gastheer-parasiet interacties vanwege hun hoge gastheerspecificiteit. De kieuwparasiet *Cichlidogyrus* in het bijzonder is een goede kandidaat voor het bevorderen van soortvorming in de gastheer, die vanwege het hoge aantal soorten, die morfologisch van elkaar verschillen, een hoge gastheerspecificiteit laat zien (Pariselle et al., 2003; Vanhove & Huyse, 2015; Vanhove et al., 2015). Daarom heb ik *Cichlidogyrus* geïdentificeerd tot soortniveau.

DEZE THESIS

In deze thesis onderzoek ik of parasieten bijdragen aan soortvorming in gastheren in cichliden uit het Victoriameer of deze soortvorming aandrijven. Met dit doel heb ik de infectie door macroparasieten geanalyseerd in een grote gemeenschap van 17 sympatrische, geradieerde cichlidensoorten en twee soorten die takken waarbinnen geen soortvorming heeft plaatsgevonden vertegenwoordigen (**hoofdstukken 2 en 5)**, als ook de verschillen in infectie tussen vier varienten van blauwe en rode *Pundamilia*-paren die variëren in hun mate van genetische differentiatie (**hoofdstukken 3 en 4**). Vissen waren geïnfecteerd door vijf genera kieuwparasieten (*Cichlidogyrus* spp., *Gyrodactylus sturmbaueri*, *Lamproglena monodi*, *Ergasilus lamellifer*, glochidia-larven van tweekleppigen) en twee endoparasieten in de buikholte (rondwormen, zuigwormen). *Ciclidogyrus* van het Victoriameer zijn grotendeels onbekend, ondanks het feit dat het goede kandidaten zijn voor het bevorderen van soortvorming in cichliden. Daarom heb ik deze parasieten morfologisch geïdentificeerd tot het soortniveau. Infectiepatronen zijn geanalyseerd op het niveau van parasitaire genera voor twee bemonsteringsjaren (2010 en 2014) en binnen het geslacht *Cichlidogyrus* voor één bemonsteringsjaar (2014).

Verschillen in infectie op het niveau van geslacht van parasieten

In **hoofdstukken 2 en 3** testte ik twee voorwaarden voor parasiet-gefaciliteerde soortvoming: *i)* verschillen in infectie tussen soorten en *ii)* consistentie in de richting van parasiet-gefaciliteerde selectie over de tijd (Karvonen & Seehausen, 2012). Zeventien sympatrische gastheersoorten van Makobe en vier paren van blauwe en rode vormen van *Pundamilia* van vier lokaties verschilden in parasitaire infecties, zowel wat betreft de aantallen parasieten als de diversiteit van parasieten, consistent met divergente parasiet- gefaciliteerde selectie. Deze verschillen waren grotendeels consistent tussen de twee bemonsteringsjaren binnen alle bemonsterde soorten van de radiatie en binnen reproductief geïsoleerde zustersoorten, en ondersteunen de voorwaarde voor parasiet-gefaciliteerde soortvorming van consistentie over de tijd.

In **hoofdstuk 3** onderzocht ik of verschillen in infectie tussen blauwe en rode soorten *Pundamilia*, afkomstig van vier lokaties, co-variëren met de mate van genetische of geografische afstand tussen de soorten. Sympatrische blauwe en rode *Pundamilia* verschilden zelfs bij weinig genetische differentiatie in infectieprofielen, maar deze verschillen waren alleen statistisch significant in het meest gedifferentieerde soorten-paar. Vergelijkingen van alle soorten-paren (sympatrisch en allopatrisch) onthulden dat de mate van verschil in de gemeenschap van parasieten toenam met toenemende genetische afstand binnen paren, rekening houdend met geografische afstand tussen eilanden. Deze resultaten suggereren dat soortverschillen in infectie afhangen van de mate van genetische differentiatie tussen gastheren: verschillen in infectie stapelen zich op met toenemende genetische divergentie tussen gastheren, in plaats van dat de verschillen voorafgaan aan genetische divergentie. Parasieten kunnen dus bijdragen aan differentiatie tussen gastheersoorten maar drijven het proces niet. De positieve correlatie tussen differentiatie in infectie en genetische differentiatie op het niveau van het geslacht van de parasieten was te zien in beide bemonsteringsjaren, en ondersteunt dus de consistentie van parasiet-gefaciliteerde selectie over de tijd.

Hoewel de intensiteit van sommige parasieten geassocieerd was met waterdiepte, kon de variatie in infectie tussen gastheersoorten verklaard worden door waterdiepte en trofische specialisatie alleen (**hoofdstukken 2 en 3**). Dit suggereert dat andere intrinsieke soorteigenschappen (zoals immuniteit en genetische vatbaarheid) ook een rol spelen. In **hoofdstuk 4** onderzocht ik de bijdrage van intrinsieke eigenschappen van gastheren aan de variatie in infecties, door infectie te onderzoeken onder laboratoriumomstandigheden met een uniforme blootstelling aan parasieten. Ik vergeleek infectiepatronen tussen wild-gevangen een eerste-generatie, in het laboratorium gekweekte, gastheren behorend tot één van de *Pundamilia*-paren uit hoofdstuk 3, alsmede in laboratorium-gekweekte interspecieke hybride gasheren. Zowel het voorkomen als de hoeveelheid van drie van de meest voorkomende ectoparasieten was vergelijkbaar tussen het laboratorium en het veld. Zoals beschreven in hoofdstuk 3 verschilden de twee soorten in infectie in het wild, echter was dit onder laboratoriumomstandigheden, waar vissen sommige soort-specifieke ecologische eigenschappen (zoals diepte en dieet-voorkeur) niet tot expressie kunnen brengen, niet het geval. Dit duidt erop dat variatie in infectie vooral door extrinsieke effecten wordt veroorzaakt, in plaats van genetisch gebaseerde soortverschillen in immuniteit. Het is onwaarschijnlijk dat verschillen in immuun-eigenschappen al in vroege stadia van soortvorming ontwikkelen, aangezien dit *Pundamilia*-paar genetisch slechts zwak is gedifferentieerd, wat consistent is met een bijdrage van parasieten aan de divergentie van *Pundamilia*.

Hybriden verschilden niet in infectie van beide ouder-soorten (allen eerste generatie laboratorium-gekweekt, **hoofdstuk 4**), inconsistent met het scenario een lagere fitness in hybriden die parasiet-gefaciliteerde soortvorming zou kunnen bevorderen. Hybriden zijn ondanks dat echter zeldzaam in het veld, waarschijnlijk vanwege soort-assortatieve paring. Het

de afwezigheid van een een lagere fitness in hybriden suggereert dat assortative paring wordt gedreven door andere ecologische factoren.

De twee *Pundamilia* soorten zijn in het wild vanwege scheiding over waterdiepte aangepast aan verschillende visuele omgevingen: blauwe vormen bewonen een omgeving met een breed lichtspectrum, terwijl rode vormen leven in een omgeving met een rood-verschoven spectrum. Deze twee visuele omgevingen werden in laboratorium nagebootst. Wanneer de gasheren opgroeien in de niet-natuurlijke omgeving is hun overlevingkans lager (Maan et al., 2017), en zou infectie door parasieten hoger kunnen zijn. Dit was echter niet waargenomen: infectie door parasieten verschilde niet tussen natuurlijke en niet-natuurlijke licht, wat suggereert dat een visuele 'mismatch' in lichtomgeving de vatbaarheid van gastheren voor parasieten niet verhoogt.

Verschillen in infectie op het niveau binnen het geslacht *Cichlidogyrus*

De samenstelling van de gemeenschap van *Cichlidogryrus* morfosoorten was vergelijkbaar tussen de bemonsterde soorten die deel uitmaken van Victoriameer-radiatie (**hoofdstuk 2**). Dit ondersteunt de idee dat *Cichlidogyrus* een rol speelt in de diversificatie van gastheren niet, omdat in dat geval gedivergeerde radiatieleden soort-specifieke resistentie zouden hebben ontwikkeld die leidt tot divergentie in infectie. De samenstelling van de *Cichlidogryrus* morfosoorten-gemeenschap verschilde daarentegen wel tussen de drie grote lijnen van gastheren – de radiatie-tak en de twee oudere takken vertegenwoordigd door *A. alluaudi* en *Ps. multicolor*. Morfosoorten die de ene tak infecteerden, infecteerden zelden de andere takken, ondanks dat de gastheren volledig sympatrisch zijn, wat een gelegenheid voor gastheerspecialisatie suggereerdt (hoewel radiatie-soorten momenteel geen verschillende bronnen voor *Cichlidogyrus*-morfosoorten vertegenwoordigen).

Er was geen geleidelijke toename in de mate van ongelijkheid in de gemeenschap van *Cichlidogyrus* met toenemende mate van genetische differentiatie van de *Pundamilia*-paren (**hoofdstuk 3**). De samenstelling van de *Cichlidogyrus* gemeenschap verschilde alleen in het reproductief geïsoleerde *Pundamilia*-paar van Makobe. Dit duidt erop dat *Cichlidogyrus* de differentiatie in *Pundamilia* niet drijft, en suggereert dat verschillen in infectie ontstaan wanneer gastheren al een bepaalde mate van divergentie hebben bereikt, in plaats van dat *Cichlidogyrus* een rol speelt in de vroege stadia van gastheer diversificatie. Bij elkaar genomen wijzen deze resultaten erop dat morfosoorten van *Cichlidogyrus* niet bijdragen aan gastheer differentiatie.

Geen geografisch patroon in verschillen in infectie van soorten

De kans om geïnfecteerd te raken en de aantallen parasieten die gastheren infecteerden verschilde tussen locaties. De hoogste infectieniveaus door nematoden (die vaak door vogels worden overgedragen) werden bijvoorbeeld waargenomen rond het eiland Makobe, waar grote

populaties aalscholvers en zilverreigers te vinden zijn. In moerassige locaties met weinig vissoorten en individuen waren de hoeveelheden parasieten over het algemeen laag vergeleken met rotsige eiland met relatief grote cichlidenpopulaties. Ondanks zulke geografishe variatie in infectieniveaus waren verschillen in infectie tussen soorten consistent over de verschillende locaties (**hoofdstukken 2 en 3**). Dit patroon was te zien voor losse parasieten (bijvoorbeeld, rode vormen van *Pundamilia* huisvesten consistent meer *L. monodi* en *E. lamillifer* dan de blauwe varianten), maar ook op het niveau van de samenstelling van de hele gemeenschap van parasieten (een toename in geografische afstand tussen populaties viel niet samen met een toename in ongelijkheid van de gemeenschap van parasieten). Het ontbreken van een geografisch patroon in verschillen in infectie van sympatrische soorten zou een scenario van parasiet-gefaciliteerde van divergentie kunnen ondersteunen, omdat differentiatie in infectie het gevolg kan zijn van intrinsieke eigenschappen van de gastheer (inclusief resistentie).

Segregatie van parasiet-microhabitats

In **hoofdstuk 5** analyseerde ik de verspreiding van de microhabitats van parasieten over de kieuwen, om te beoordelen of dit een andere as van divergentie in infectie zou kunnen uitmaken. De twee meest voorkomende ectoparasiet taxa (*Cichlidogyrus* spp., *L. monodi*) en de *Cichlidogyrus* (*C. nyanza*, *C. furu*)-morfosoorten hadden niet-random microhabitat-distributies die verschilde tussen de gastheersoorten, wat suggereert dat de interactie van dezelfde parasiet met zijn gastheer zou kunnen verschillen tussen verschillende gastheersoorten. Dit zou een gelegenheid kunnen bieden voor parasiet-gefaciliteerde gastheerdifferentiatie. De selectie van microhabitat vertegenwoordigd een andere as van heterogeniteit in infecties die meer verschillen zou kunnen blootleggen dan tellingen van parasieten. Het is daarom nuttig dat deze factor in toekomstige studies wordt meegenomen. De verhoudingen tussen verschillende parasieten verschilden niet tussen gastheersoorten. Dit zou in monogeneanen verklaard kunnen worden door toenemende paringskansen (aangezien zij op de gastheer reproduceren), in roeipootkreeftjes door de blootstelling van eieren aan water (aangezien de meeste roeipootkreeftjes op zo'n manier zijn bevestigd dat legsels buiten de kieuwfilamenten zijn blootgesteld).

Dynamiek binnen gastheerparasieten

In **hoofdstuk 5** observeerde ik positieve correlaties tussen de hoeveelheid ectoparasitaire taxa en negatieve correlaties tussen morfosoorten van *Cichlidogyrus*. Positieve relaties kunnen op verschillende manieren worden uitgelegd: *i)* het zijn ware synergetische interacties, mogelijk vanwege de overeenkomst in de antigenen van parasieten die de uitbuiting van immunomodulatie door andere parasieten mogelijk maken; *ii)* ze zijn het gevolg van de associatie met dezelfde ecologische specialisatie van de gastheer. Negatieve relaties zouden veroorzaakt kunnen worden door competitie, mogelijk gerelateerd aan de fylogenetische

verwantschap van parasieten of aan de overeenkomsten in benodigde bronnen. De richting en mate van parasitaire interacties verschilde niet tussen gastheren, wat erop wijst dat intrinsieke eigenschappen van gastheersoorten geen invloed hebben op de relaties tussen parasieten, wat inconsistent is met gastheer specificiteit.

Ik heb ook de verschillen tussen gastheersoorten in reproductieve activiteit van roeipootkreeftjes onderzocht (gemeten in de proportie van legsel-dragende vrouwtjes), en vond geen verschillen tussen wild-gevangen soorten (**hoofdstuk 5**) en tussen de twee lab-gekweekte soorten van *Pundamilia* en hun interspecifieke hybriden (**hoofdstuk 4**). Dit wijst op het ontbreken van gastheerspecificiteit in de reproductieve activiteit van roeipootkreeftjes, hoewel roeipootkreeftjes variëren in zowel aantallen als het algemeen voorkomen van infecties.

CONCLUSIE

In deze thesis vond ik ondersteuning voor een bijdrage van parasieten in divergentie tussen gastheren, maar niet in het initiëren van deze divergentie. Ten eerste, parasieten zijn niet-random verspreid op tenminste drie niveaus – kieuw-microhabitat, gastheersoort, gastheertak – wijzend op gastheer specialisatie en een mogelijkheid voor heterogene, parasiet-gefaciliteerde selectie. Ten tweede, soortverschillen in infectie waren consistent over de tijd, in lijn met de voorwaarden voor parasiet- gefaciliteerde soortvorming. Ten derde, wanneer gastheersoorten beginnen te divergeren in ecologie, begint ook de opstapeling van verschillen in hun gemeenschap van parasieten, wat erop wijst dat de differentiatie in infectie een bijproduct is van divergentie, in plaats van het tegenovergestelde.

Riassunto in italiano

Translation by Tiziana P Gobbin

SPECIAZIONE MEDIATA DA PARASSITI

La speciazione – la formazione di nuove specie – è stata definita da Darwin come il "mistero dei misteri" più di un secolo fa. Da allora, i meccanismi di speciazione sono stati studiati intensamente e molti progressi sono stati fatti. Gli adattamenti a fattori biotici e abiotici possono causare la speciazione come effetto secondario e, tra i fattori biotici, i parassiti possono essere un importante agente di selezione. Tuttavia, alcuni meccanismi di speciazione rimangono poco esplorati. In particolare, l'inizio della divergenza non è ancora ben compreso. In questa tesi, indago quando e come la selezione divergente mediata da parassiti contribuisca al processo di speciazione.

I parassiti costituiscono una fonte di selezione ecologica molto diffusa (Poulin & Morand, 2000; Schmid-Hempel, 2013), che potrebbe potenzialmente fungere da motore della speciazione (Schluter, 1996, 2000b; Rundle & Nosil, 2005; Maan & Seehausen, 2011). Per definizione, i parassiti impongono costi di fitness ai loro ospiti (ad esempio riduzione della crescita, della riproduzione e della sopravvivenza, Agnew et al., 2000; Lafferty & Kuris, 2009; Segar et al., 2018). Gli ospiti si adattano ai parassiti evolvendo una resistenza, una tolleranza o tramite comportamenti di evitamento. A loro volta, i parassiti si adattano in risposta agli ospiti eludendo o sopprimendo l'immunità dell'ospite. Ciò porta a una dinamica co-evolutiva di adattamento e contro adattamento (Decaestecker et al., 2007).

Le popolazioni di ospiti che occupano diverse nicchie ecologiche possono essere esposte a diverse quantità e specie di parassiti, risultando potenzialmente in differenti selezioni mediate da parassiti (Knudsen et al., 2004; Pegg et al., 2015; Hablützel et al., 2017; Hayward et al., 2017) anche in simpatria. Ciò può indurre le popolazioni di ospiti a sviluppare differenti adattamenti contro le minacce dei parassiti locali. Tali risposte adattative possono portare a una crescente differenza nell'infezione parassitaria tra le popolazioni di ospiti. Se queste differenze vengono mantenute nel tempo, allora la selezione mediata dai parassiti agisce continuamente nella stessa direzione, promuovendo la divergenza dell'ospite. Le fluttuazioni temporali stocastiche o dipendenti dalla frequenza nell'abbondanza dei parassiti potrebbero causare variazioni nella forza della selezione mediata da parassiti, ma la divergenza viene comunque promossa fintanto che la direzione della selezione viene mantenuta (**Fig. 11.1**).

Queste differenze di infezione stabili nel tempo possono portare alla differenziazione genetica tra le popolazioni di ospiti ed infine instaurare o rafforzare l'isolamento riproduttivo tra loro (Hamilton & Zuk, 1982; Landry et al., 2001; Nosil et al., 2005; Maan et al., 2008; Eizaguirre et al., 2011). Inoltre, l'isolamento riproduttivo potrebbe essere rafforzato dalla selezione contro gli ibridi e gli immigrati (tramite infezione più elevata), dalla scelta del partner immuno-mediata (scegliendo partner che forniscono immunità localmente adattata) o scelta del partner mediata

dai parassiti (scegliendo partner sani). In alternativa, la selezione divergente mediata da parassiti può rafforzare la differenziazione dell'ospite una volta che un certo grado di isolamento riproduttivo è già stato raggiunto attraverso altri meccanismi (Haldane, 1949; Price et al., 1986; Karvonen & Seehausen, 2012).

Lo studio della speciazione mediata da parassiti può essere problematico a causa della natura bidirezionale dell'interazione ospite-parassita – per cui bisogna determinare quale dei due stia conducendo la diversificazione – e a causa del coinvolgimento di altri fattori ecologici, per cui bisogna distinguere gli effetti dei parassiti da quelli di altri potenziali fattori di diversificazione. A tal fine, le popolazioni di ospiti nelle prime fasi della speciazione forniscono un buon modello di studio. In questa tesi, mi avvalgo della giovane radiazione adattativa dei pesci ciclidi nel Lago Vittoria per studiare il ruolo dei parassiti nella speciazione degli ospiti.

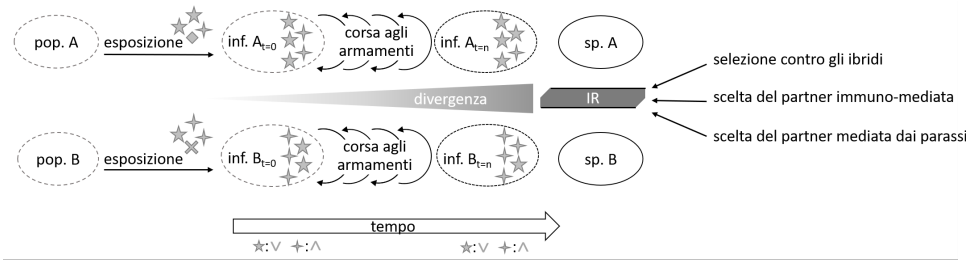


Figura 11.1
Speciazione mediata da parassiti. Due popolazioni di ospiti in grado di ibridarsi occupano due diverse nicchie ecologiche (A e B) e sono esposte a diverse quantità e specie di parassiti (simboli), risultando in due diverse infezioni. Ciascuna popolazione di ospiti evolve adattamenti contro i parassiti locali, impegnandosi in una corsa agli armamenti evolutiva. La direzione della differenza nell'infezione rimane costante nel tempo, nonostante le fluttuazioni nell'abbondanza complessiva (★ maggiore nella popolazione A rispetto a B, ✦ minore in A rispetto a B). La divergenza nelle difese contro i parassiti porta all'isolamento riproduttivo (IR) tra le popolazioni ospiti – che può essere rafforzato tramite selezione contro ibridi e/o immigrati, e/o scelta del partner immuno-mediata, e/o scelta del partner mediata dai parassiti – risultando così in due distinte specie di ospiti.

I CICLIDI DEL LAGO VITTORIA E I LORO PARASSITI

La radiazione adattativa dei pesci ciclidi del lago Vittoria è ideale per studiare la speciazione mediata da parassiti, a causa della sua giovane età, della diversità ecologica interspecifica e della differenziazione genetica relativamente debole. Appena 14'600 anni fa, il lago si è nuovamente riempito dopo essere stato asciutto per migliaia di anni (Johnson et al., 1996; Stager & Johnson, 2008). Due lignaggi fluviali hanno poi colonizzato il lago, formando una popolazione ibrida

(Seehausen et al., 2003; Meier et al., 2017a) e fornendo la variazione genetica che ha facilitato la rapida speciazione adattativa (Seehausen, 2004; Salzburger, 2018). Pertanto, la maggior parte dei ciclidi del Lago Vittoria si è evoluta *in situ* dopo tale periodo di siccità (Johnson et al., 2000; Stager & Johnson, 2008; Wagner et al., 2013; Meier et al., 2017a). Nel Lago Vittoria, i membri della radiazione co-esistono con lignaggi più antichi e lontanamente imparentati che non hanno speciato dopo aver colonizzato il lago: *Astatoreochromis alluaudi* (Pellegrin, 1904), *Pseudocrenilabrus multicolor* (Schöller, 1903), *Oreochromis variabilis* (Boulenger, 1906) e *Oreochromis esculentus* (Graham, 1928). Questi forniscono un utile termine di paragone per studiare il motivo per cui alcuni lignaggi hanno speciato e altri invece no.

Studi precedenti suggeriscono che la speciazione mediata da parassiti potrebbe avvenire nei ciclidi, a causa dell'elevata diversità delle nicchie ecologiche (Fryer & Iles, 1972; Wagner et al., 2012a), della potenzialmente alta trasmissibilità delle malattie dovuta all'elevata densità dei pesci (Ribbink et al., 1983; Fenton et al., 2002), dell'associazione tra parassitismo ed espressione dei segnali sessuali (Taylor et al., 1998; Maan et al., 2006b) e della rapida evoluzione dei geni MHC (legati alla risposta immunitaria, Blais et al., 2007). Inoltre, ci sono prove a sostegno della co-evoluzione tra i ciclidi del lago Tanganica e i monogenei che parassitano le loro branchie (Vanhove et al., 2015).

Il lago Vittoria ospita anche delle repliche di speciazione che si trovano in diversi stadi, il che consente di valutare quando – durante il processo di speciazione – si manifestano delle differenze nell'infezione. *Pundamilia pundamilia* (Seehausen et al., 1998), di color blu, e *Pundamilia nyererei* (Witte-Maas e Witte, 1985), di color rosso, sono due ciclidi strettamente imparentati che co-abitano le isole rocciose nella parte sud-orientale del lago. In alcune località queste due specie si sono ibridate e poi hanno nuovamente speciato formando delle coppie blu e rosse simili alle specie d'origine (Meier et al., 2017b; Meier et al., 2018). Attraverso le varie località, le forme blu e rosse variano nel loro grado di differenziazione genetica (Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018), differenziazione morfologica (van Rijssel et al., 2018a), differenziazione nell'adattamento visivo (Carleton et al., 2005; Seehausen et al., 2008; Wright et al., 2019) e nella frequenza di ibridazione (Seehausen, 1996a; Seehausen et al., 2008; Meier et al., 2017b; Meier et al., 2018). La variazione di questi tratti è associata alla trasparenza dell'acqua.

I parassiti vivono a spese degli ospiti, imponendo loro un costo di fitness. Il ciclo vitale del parassita può comportare una o più specie ospiti (in cui l'ospite finale alberga gli stadi riproduttivi) e può comprendere anche stadi di vita libera (spesso uova o larve). I ciclidi ospitano numerosi taxa di macro-parassiti: monogenei (parassiti delle branchie, ma alcuni generi infettano il tratto digestivo o la vescica, con un ciclo di vita diretto), copepodi (parassiti delle branchie o della pelle, con un ciclo di vita diretto), molluschi bivalvi (parassiti delle branchie o della pelle, con un ciclo di vita diretto), nematodi (endoparassiti, di cui i pesci sono spesso ospiti

intermedi), trematodi (endoparassiti con almeno due ospiti intermedi). I monogenei sono di grande interesse nello studio delle interazioni ospite-parassita a causa della loro elevata specificità per le specie ospite. In particolare, il monogeneo delle branchie *Cichlidogyrus* è un buon candidato per la promozione della speciazione degli ospiti, grazie al suo elevato numero di specie che differiscono nella morfologia e all'alta specificità (Pariselle et al., 2003; Vanhove & Huyse, 2015; Vanhove et al., 2015).

QUESTA TESI

In questa tesi ho investigato se i parassiti iniziassero o contribuissero alla speciazione dei ciclidi del lago Vittoria. A tal fine, ho analizzato l'infezione da macro-parassiti di una grande comunità simpatrica di 17 specie di ciclidi appartenenti alla radiazione e due specie rappresentanti due lignaggi che non hanno mai speciato (**capitoli 2 e 5**), nonché le differenze di infezione tra quattro repliche di coppie blu e rosse di *Pundamilia* che variano nel grado di differenziazione genetica (**capitoli 3 e 4**). I pesci erano infettati da cinque generi di parassiti delle branchie (*Cichlidogyrus* spp., *Gyrodactylus sturmbaueri*, *Lamproglena monodi*, *Ergasilus lamellifer*, larve glochidia di bivalvi) e due endoparassiti della cavità addominale (nematodi, trematodi). Nonostante siano degli ottimi candidati per promuovere la speciazione dei ciclidi, i *Cichlidogyrus* del lago Vittoria sono perlopiù sconosciuti. Pertanto, li ho identificati morfologicamente a livello di specie. I pattern di infezione sono stati analizzati a livello di genere dei parassiti in due diversi anni di campionamento (2010 e 2014) e a livello di *Cichlidogyrus* in un solo anno di campionamento (2014). Dato che i risultati differiscono a seconda del livello analizzato, essi vengono presentati separatamente.

Differenze di infezione a livello di genere dei parassiti

Nei **capitoli 2 e 3** ho testato due prerequisiti per la speciazione mediata da parassiti: *i)* le specie ospiti differiscono nell'infezione parassitaria e *ii)* la direzione della selezione mediata da parassiti rimane consistente nel tempo (Karvonen & Seehausen, 2012). Diciassette specie ospiti simpatriche a Makobe e quattro coppie di *Pundamilia* blu e rossi in quattro località differivano nella loro infezione parassitaria, sia in termini di abbondanza che di diversità, coerentemente con la selezione divergente mediata da parassiti. Queste differenze di infezione erano perlopiù coerenti tra i due anni di campionamento, sia tra i membri della radiazione campionati che nella coppia di specie ospiti riproduttivamente isolata, supportando il prerequisito di continuità temporale della selezione mediata da parassiti.

Nel **capitolo 3**, ho verificato se le differenze di infezione tra le forme blue e rosse di *Pundamilia*, da quattro località, co-variano col grado di distanza genetica o geografica presente tra loro. Solamente la coppia blu-rossa con la più alta differenziazione genetica differiva nei profili di

infezione. Il paragone tra tutte le coppie (simpatriche e allopatriche, di colore uguale o diverso) ha rivelato che il grado di dissomiglianza della comunità parassitaria aumenta con l'aumentare della distanza genetica all'interno delle coppie, tenendo conto della distanza geografica tra le località. Questi risultati suggeriscono che le differenze di infezione tra specie ospiti dipendono dal grado di differenziazione genetica dell'ospite: le differenze di infezione si accumulano con l'aumentare della divergenza genetica dell'ospite, piuttosto che precederla. Pertanto, i parassiti possono contribuire alla differenziazione delle specie ospiti ma non la iniziano. La correlazione positiva tra differenziazione delle infezioni e differenziazione genetica a livello del genere di parassita è stata osservata in entrambi gli anni di campionamento, supportando la continuità temporale della selezione mediata da parassiti.

Sebbene l'intensità di alcuni parassiti sia associata alla profondità dell'acqua, la variazione dell'infezione tra le specie ospiti non è completamente spiegata né dalla profondità né dalla specializzazione trofica (**capitoli 2 e 3**). Ciò suggerisce che anche altre proprietà intrinseche della specie ospite (come immunità e suscettibilità genetica) svolgono un ruolo. Nel **capitolo 4**, ho studiato il contributo delle proprietà intrinseche della specie ospite alla variazione dell'infezione, valutando le differenze di infezione tra due specie ospite mantenute in laboratorio con esposizione uniforme ai parassiti. Ho esaminato i pattern di infezione di una delle coppie di *Pundamilia* del capitolo 3, confrontando le differenze interspecifiche di infezione osservate in natura con quelle delle stesse specie allevate in laboratorio (prima generazione) e tra queste e gli ibridi interspecifici di prima generazione allevati in laboratorio. Sia in natura che in laboratorio, tre degli ectoparassiti più comuni presentavano livelli di prevalenza e abbondanza paragonabili. Le due specie differivano nell'infezione in natura ma non in laboratorio, condizione in cui i pesci non possono esprimere alcuni tratti ecologici specie-specifici (ad es. preferenze di profondità e dieta). Ciò indica che la variazione di infezione è dovuta principalmente agli effetti estrinseci piuttosto che a differenze genetiche legate all'immunità. Poiché questa coppia di *Pundamilia* è debolmente differenziata geneticamente, è improbabile che le differenze nei tratti immunitari si siano evolute già nelle prime fasi della speciazione. Ciò non è compatibile con un contributo dei parassiti nella divergenza tra *Pundamilia*.

I livelli di infezione degli ibridi non differivano da quelli delle specie parentali (unicamente individui di prima generazione allevati in laboratorio, **capitolo 4**), in contrasto con l'atteso svantaggio ibrido che avrebbe favorito la diversificazione mediata da parassiti. Nonostante ciò gli ibridi sono rari in natura, probabilmente a causa dell'accoppiamento assortativo per specie. La mancanza di svantaggio ibrido suggerisce che l'accoppiamento assortativo è guidato da altri fattori ecologici.

In natura, a causa della segregazione di profondità, le due specie di *Pundamilia* sono adattate a diversi ambienti visivi: le forme blu vivono in condizioni di luce ad ampio spettro, mentre le forme rosse in condizioni di luce nello spettro rosso. Queste due condizioni visive sono state ricreate in

laboratorio. Una discordanza tra specie e ambiente visivo coincide con una minore sopravvivenza (Maan et al., 2017) e potrebbe coincidere anche con una più alta infezione da parassiti. Ciò non è però stato osservato: l'infezione non differiva tra ospiti allevati in condizioni di luce naturale e innaturale, suggerendo che una mancata corrispondenza tra specie e ambiente visivo non aumenta la suscettibilità dell'ospite alle infezioni parassitarie.

Differenze di infezione a livello di specie di *Cichlidogyrus*

Contrariamente a quanto osservato a livello di generi di parassiti, la composizione della comunità di morfospecie di *Cichlidogyrus* non differiva tra le specie ospite campionate appartenenti alla radiazione del Lago Vittoria (**capitolo 2**). Ciò non supporta un ruolo di *Cichlidogyrus* nella diversificazione dell'ospite, poiché tale scenario prevedeva che i membri della radiazione, essendosi diversificati di recente, evolvessero una resistenza specie-specifica legata alla divergenza dell'infezione. Invece, la comunità di *Cichlidogyrus* differiva tra i tre lignaggi di ciclidi: il lignaggio delle radiazioni e i due lignaggi più antichi rappresentati da *A. alluaudi* e *Ps. multicolor*. Nonostante la simpatria delle specie ospiti, le morfospecie di *Cichlidogyrus* che infettano un lignaggio raramente ne infettano un altro. Questo suggerisce che vi sia un'opportunità di specializzazione dell'ospite (sebbene attualmente i membri della radiazione non rappresentino risorse diverse per *Cichlidogyrus*).

Focalizzandosi sulle coppie di specie di *Pundamilia*, non c'è un graduale aumento nel grado di dissomiglianza nella comunità di *Cichlidogyrus* all'aumentare della differenziazione genetica tra le popolazioni ospiti (**capitolo 3**), contrariamente a quanto osservato a livello di generi di parassiti. Così come osservato a livello di generi di parassiti, l'unica coppia in simpatria che differiva nell'infezione delle morfospecie di *Cichlidogyrus* è quella riproduttivamente isolata a Makobe. Ciò indica che *Cichlidogyrus* non sta guidando la differenziazione in *Pundamilia*. Indica invece che le differenze nell'infezione sorgono quando gli ospiti hanno già raggiunto un certo grado di divergenza, contrariamente a quanto atteso se *Cichlidogyrus* contribuisse nelle prime fasi della diversificazione dell'ospite. Insieme, questi risultati suggeriscono che le morfospecie di *Cichlidogyrus* non contribuiscono alla differenziazione dell'ospite.

Nessun pattern geografico nelle differenze di infezione tra specie

Le probabilità di infettarsi e il numero parassiti che infettano gli ospiti variano tra le diverse località geografiche. Ciò può essere spiegato dalle differenze ecologiche tra tali località. Ad esempio, i più alti livelli di infezione da nematodi (spesso trasmessi dagli uccelli) sono stati osservati sull'isola di Makobe, abitata da grandi popolazioni di cormorani ed egrette. L'abbondanza di parassiti era generalmente bassa nella località paludosa con poche specie e individui di pesci, rispetto alle isole rocciose con relativamente grandi popolazioni di ciclidi. Nonostante tale variazione geografica nei livelli di infezione, le differenze tra le specie ospiti

nell'infezione erano consistenti tra le varie località (**capitoli 2 e 3**). Questo schema è stato osservato per i singoli taxa di parassiti (ad esempio le forme rosse di *Pundamilia* ospitavano costantemente più *L. monodi* ed *E. lamellifer* rispetto alle forme blu) ma anche a livello di composizione della comunità di parassiti (un aumento della distanza geografica tra le popolazioni non coincideva con un aumento della dissomiglianza della comunità parassitaria). Una mancanza di pattern geografico nelle differenze di infezione tra specie simpatriche può supportare uno scenario di diversificazione simpatrica mediata da parassiti, poiché la differenziazione nell'infezione può derivare da tratti intrinseci dell'ospite (inclusa la resistenza).

Segregazione nei microhabitat dei parassiti

Nel **capitolo 5** ho analizzato la distribuzione dei microhabitat dei parassiti sulle branchie, per valutare se ciò potesse costituire un altro asse di divergenza nell'infezione. I due taxa più abbondanti di ectoparassiti (*Cichlidogyrus* spp., *L. monodi*) e di morfospecie di *Cichlidogyrus* (*C. nyanza*, *C. furu*) presentavano una distribuzione di microhabitat non casuale che differiva tra le specie ospiti, suggerendo che lo stesso parassita potrebbe interagire in modo differente con differenti specie ospite. Ciò può fornire opportunità di differenziazione dell'ospite mediata da parassiti. La selezione di microhabitat rappresenta un altro asse di eterogeneità nell'infezione che può rivelare più differenze di quanto faccia il semplice conteggio dei parassiti, quindi è auspicabile che venga incluso anche in studi futuri. Le relazioni interspecifiche tra parassiti non differivano tra le specie ospiti. Nei monogenei ciò può essere spiegato da maggiori opportunità di accoppiamento (poiché si riproducono sull'ospite); mentre nei copepodi ciò può essere spiegato dall'esposizione delle uova al flusso d'acqua (poiché la maggior parte dei copepodi è attaccata alla branchia in modo tale da esporre le uova all'esterno dei filamenti branchiali).

Dinamiche parassitarie nell'ospite

Nel **capitolo 5** ho osservato correlazioni positive tra l'abbondanza di ectoparassiti (a livello di genere) e correlazioni negative tra l'abbondanza di morfospecie di *Cichlidogyrus*. Le relazioni positive possono essere spiegate in diversi modi: *i)* sono vere interazioni sinergiche, potenzialmente derivanti dalla somiglianza antigenica del parassita che consente lo sfruttamento dell'immunomodulazione da parte dell'altro parassita *ii)* risultano dal fatto che entrambi i parassiti sono associati alla stessa specializzazione ecologica dell'ospite. Le relazioni negative possono essere dovute alla concorrenza, eventualmente connessa alla relazione filogenetica dei parassiti o alla somiglianza nei requisiti delle risorse. La direzione e la forza delle interazioni tra parassiti non differivano tra le specie ospiti, suggerendo che i tratti intrinseci delle specie ospiti non influenzino le relazioni con i parassiti, in contrasto con la specificità dell'ospite.

Ho anche esplorato le differenze nell'attività riproduttiva dei copepodi (misurata come la proporzione di femmine adulte munite di uova) tra le specie ospiti. Non ho osservato differenze

né tra le specie catturate in natura (**capitolo 5**) né tra le due specie di *Pundamilia* allevate in laboratorio e i loro ibridi interspecifici (**capitolo 4**). Ciò suggerisce che non vi è nessuna specificità dell'ospite nell'attività riproduttiva del copepode, sebbene esso vari nei livelli di infezione (prevalenza e abbondanza).

CONCLUSIONE

In questa tesi, ho trovato evidenze a supporto del ruolo dei parassiti nel contribuire alla divergenza dell'ospite, ma non nell'iniziarla. Primo, i parassiti sono distribuiti in modo non casuale ad almeno tre livelli – microhabitat delle branchie, specie ospiti, lignaggi ospiti – indicando una specializzazione del parassita e un'opportunità di selezione eterogenea mediata da parassiti. Secondo, le differenze tra specie nell'infezione sono temporalmente coerenti, in linea con i prerequisiti per la speciazione mediata da parassiti. Terzo, quando le specie ospiti iniziano a divergere in ecologia, esse iniziano anche ad accumulare differenze nelle comunità di parassiti, suggerendo che la differenziazione nell'infezione è una conseguenza della divergenza piuttosto che il contrario.

Deutsche Zusammenfassung

Translation by Philip Kohlmeier

PARASITENVERMITTELTE SPEZIATION

Parasiten stellen, indem sie die Fitness ihrer Wirte reduzieren (z.B. Agnew et al., 2000; Lafferty und Kuris, 2009; Segar et al., 2018), eine weit verbreitete Ursache ökologischer Selektion dar (Poulin und Morand, 2000; Schmid-Hempel, 2013), die potentiell zu Speziation führen kann (Maan und Seehausen, 2011; Rundle und Nosil, 2005; Schluter, 1996, 2000). Wirte passen sich an Parasiten an, indem sie Resistenz, Toleranz oder Verhaltensvermeidung gegen den jeweiligen Parasiten entwickeln. Im Gegenzug entwickeln Parasiten Adaptionen, die es ihnen ermöglichen, sich der Wirtsimmunität zu entziehen oder diese zu unterdrücken. Dies führt zu einer koevolutiven Dynamik stetiger Adaption und Gegenadaption (Decaestecker et al., 2007). Selbst sympatrisch lebende Wirtspopulationen, die unterschiedliche ökologische Nischen besetzen, können unterschiedlichen Mengen und Arten an Parasiten ausgesetzt sein (Hablützel et al., 2017; Hayward et al., 2017; Knudsen et al., 2004; Pegg et al., 2015). Wenn diese Unterschiede in Rate und Ausmaß der Infektion über längere Zeiräume konstant bleiben, kann der gleichbleibende und gleichgerichtete Selektiondruck die Divergenz der Wirtspopulationen begünstigen. Solche divergierenden und zeitlich stabilen Infektionsunterschiede können schließlich zur genetischen Differenzierung zwischen den Wirtspopulationen führen und somit die reproduktive Isolation zwischen ihnen entweder herbeiführen oder verstärken (Eizaguirre et al., 2011; Hamilton und Zuk, 1982; Landry et al., 2001; Maan et al., 2008; Nosil et al., 2005). In dieser Arbeit nutze ich die evolutionär junge adaptive Radiation von Cichlidenfischen im Viktoriasee, um zu untersuchen, wann und auf welche Weise parasitenvermittelte divergente Selektion zum Speziationsprozess beiträgt.

VIKTORIABUNTBARSCHE UND IHRE PARASITEN

Die meisten Buntbarsch-Arten im Viktoriasee entwickelten sich in situ aus einem Hybridschwarm zweier Flussbewohnender Linien (Meier et al., 2017a; Seehausen et al., 2003), der den See nach seiner Wiederauffüllung vor 14.600 Jahren besiedelte (Johnson et al., 1996; Stager und Johnson, 2008). Arten, die aus Radiationen entstanden, leben hier mit Vertretern evolutionär älterer und entfernt verwandter Linien zusammen, welche nach der Kolonisierung des Sees keiner Speziation unterliefen. Im Viktoriasee finden sich zudem Zwischenstufen der Speziation, was die Möglichkeit eröffnet, den Zeitpunkt zu bestimmen, an dem innerhalb des Speziationsprozesses Unterschiede in den Infektionsmustern das erste Mal auftraten. Buntbarsche dienen zahlreichen Makroparasiten-Taxa als Wirte: Monogeneane (meist Kiemenparasiten mit einem direkten Lebenszyklus), Copepoden (Kiemen- oder Hautparasiten mit einem direkten Lebenszyklus), Muscheln (Kiemen- oder Hautparasiten mit einem direkten Lebenszyklus), Nematoden (Endoparasiten, die häufig Fische als Zwischenwirte nutzen), Trematoden (Egel und Endoparasiten mit mindestens zwei Zwischenwirten).

DIESE STUDIE

Um zu untersuchen, ob Parasiten lediglich zur Wirtsspeziation bei viktorianischen Buntbarschen beitragen oder ursächlich für Wirtsspeziation sind, analysierte ich die Makroparasiten-Infektion einer sympatrischen Gemeinschaft von 17 Buntbarschen-Arten, die aus Radiation hervorgingen, und zwei Arten, die evolutionäre Linien repräsentieren, welche keine Speziation durchliefen (**Kapitel 2 und 5**), sowie Infektionsunterschiede zwischen vier Replikaten blauer und roter Pundamilia-Paare, die sich im Ausmaß der genetischen und ökologischen Differenzierung unterscheiden (**Kapitel 3 und 4**). Die Fische können von insgesamt fünf Gattungen von Kiemenparasiten und zwei Endoparasiten in der Bauchhöhle infiziert werden. Da der Kiemenparasit *Cichlidogyrus* auf Grund seiner hohen Anzahl wirtspezifischer Arten ein vielversprechender Kandidat für die Förderung der Wirtsspeziation ist (Pariselle et al., 2003; Vanhove und Huyse, 2015; Vanhove et al., 2015), habe ich sie morphologisch bis auf Artniveau bestimmt. Die Infektionsmuster zwischen den verschiedenen Parasitengattungen wurden für zwei Stichprobenjahre (2010 und 2014) und innerhalb von *Cichlidogyrus* für ein Stichprobenjahr (2014) analysiert.

Infektionsunterschiede auf der Ebene der Parasitengattung

Siebzehn sympatrische Wirtsarten in Makobe sowie vier Paare blauer und roter *Pundamilia* an insgesamt vier Standorten zeigten Unterschiede in den Infektionsmustern (**Kapitel 2 und 3**), was die Hypothese einer divergierenden parasitenvermittelten Selektion bekräftigt. Diese Infektionsunterschiede waren zwischen den beiden Beprobungsjahren weitgehend konsistent, was die Hypothese einer zeitlichen Konsistenz bei der durch Parasiten ausgelösten Speziation unterstützt.

Infektionsunterschiede blauer und roter *Pundamilia* aus vier Standorten kovariieren mit dem Ausmaß der genetischen Differenzierung zwischen ihnen (**Kapitel 3**). Dieses Muster wurde in beiden Stichprobenjahren beobachtet, was mit der Idee einer zeitlich konstanten und durch Parasiten ausgelösten Selektion übereinstimmt. Betrachtet man nur sympatrisch lebende blau-rote Paare, so unterschied sich die Parasiteninfektion nur bei dem genetisch am stärksten differenzierten Paar. Diese Ergebnisse deuten darauf hin, dass die Infektionsunterschiede sich mit zunehmender genetischer Divergenz des Wirts akkumulieren, anstatt ihr vorauszugehen, was andeutet, dass die Parasiten zur Wirtsdivergenz beitragen können, diese aber nicht initiieren.

Obwohl der Befall mit einigen Parasiten von der Wassertiefe abhängig war, konnten die Infektionsunterschiede zwischen den Wirtsarten nicht vollständig durch die Wassertiefe und die trophische Spezialisierung erklärt werden (**Kapitel 2 und 3**). In **Kapitel 4** untersuchte ich, inwieweit ökologische Unterschiede zwischen den Wirten zur Variation der Infektionsmuster

beitragen. Hierzu verglich ich die Infektionsmuster eines der Pundamilia-Paare aus Kapitel 3 im Feld (d.h. die Wirtsarten unterscheiden sich in ihrer Ökologie) und im Labor (d.h. die Wirtsarten können keinen Präferenzen bezüglich Tiefe und Nahrung nachgehen). Die beiden Wirtsarten unterschieden sich in der Infektion in der freien Natur, nicht aber unter Laborbedingungen. Dies deutet darauf hin, dass die Variation der Infektion hauptsächlich auf ökologische Effekte und nicht auf genetisch bedingte Unterschiede in der Immunität der Spezies zurückzuführen ist. Da dieses Pundamilia-Paar genetisch schwach differenziert ist, ist es unwahrscheinlich, dass Unterschiede in den Immunmerkmalen bereits in frühen Stadien der Speziation entstanden sind, was mit der Idee eines Beitrages des Parasiten zur Divergenz von *Pundamilia* unvereinbar ist.

Unter Laborbedingungen unterschieden sich Hybriden hinsichtlich des Infektionsmusters nicht von denen der beiden elterlichen Arten (alle als erste im Labor gezüchtete Generation, **Kapitel 4**), was einem Hybridnachteil, der eine parasitenvermittelte Diversifikation fördern könnte, widerspricht. Trotzdem sind Hybride im Freiland selten, was wahrscheinlich auf die Artbezogene Paarungspräferenz zurückzuführen ist. Das Fehlen eines HJybridnachteils deutet darauf hin, dass die assortative Paarung von anderen ökologischen Faktoren angetrieben wird.

Infektionsunterschiede innerhalb von *Cichlidogyrus*

Im Gegensatz zur Gattungsebene der Parasiten, war die Zusammensetzung der *Cichlidogyrus*-Gemeinschaft zwischen den beprobten Arten, die aus der Radiation im Victoriasee hervorgegangen sind, vergleichbar (**Kapitel 2**). Dies spricht nicht für eine Rolle von *Cichlidogyrus* bei der Wirtsdiversifizierung, da erwartet werden kann, dass erst kürzlich divergierende Arten eine artspezifische Resistenz entwickeln. Stattdessen unterschied sich die Zusammensetzung der *Cichlidogyrus*-Gemeinschaft zwischen den drei Wirtslinien - der Radiations-Linie und den beiden älteren Linien, die nicht in Speziation aufgingen. Trotz ausgeprägter Sympathie des Wirts infizierten Morphospezies, die eine bestimmte Linie infizierten, selten eine andere Linie, was die Möglichkeit zur Wirtsspezialisierung bietet.

Bei Berücksichtigung aller *Pundamilia*-Paare konnte keine Korrelation zwischen dem Ausmaß der Unähnlichkeit innerhalb der *Cichlidogyrus*-Gemeinschaft und der genetischen Differenzierung des Wirts gefunden werden (**Kapitel 3**), was dem, was auf der Ebene der Gattung der Parasiten gefunden wurde, widerspricht. Im Einklang mit den Ergebnissen auf Gattungsebene der Parasiten unterschied sich lediglich das reproduktiv isolierte blau-rote Paar bezüglich der Infektion von *Cichlidogyrus*. Dies unterstützt die Schlussfolgerung, dass Infektionsunterschiede erst entstehen, wenn die Wirte bereits ein gewisses Maß an Divergenz erreicht haben, was im Widerspruch zur *Cichlidogyrus*-getriebenen Wirtsdifferenzierung steht.

Parasiten-Mikrohabitat-Segregation und Parasitendynamik innerhalb des Wirts

Die Infektionsheterogenität wird oft anhand der Anzahl der Parasiten bestimmt auch wenn andere Aspekte der Wirt-Parasit-Interaktion ebenfalls relevant sein können. In **Kapitel 5** fand ich, dass die Mikrohabitat-Verteilung der Parasiten in den Kiemen eine weitere Achse der Divergenz bei der Infektion darstellen kann, wohingegen Korrelationen zwischen den Abundanzen der Ektoparasiten-Taxa und der Fortpflanzungsaktivität der Copepoden keine Rolle spielen. Die beiden am häufigsten vorkommenden Ektoparasiten-Taxa (*Cichlidogyrus* spp., *L. monodi*) und Morphospezies von *Cichlidogyrus* (*C. nyanza*, *C. furu*) hatten eine nicht zufällige Mikrohabitat-Verteilung, die sich zwischen den Wirtsarten unterschieden, was andeutet, dass der gleiche Parasit mit verschiedenen Wirtsarten auf unterschiedliche Weise interagieren kann. Dies kann zur parasitenvermittelte Wirtsdifferenzierung führen.

Ich beobachtete positive Korrelationen zwischen den Abundanzen der jeweiligen Ektoparasiten-Taxa und negative Korrelationen zwischen den jeweiligen Morphospezies von *Cichlidogyrus*. Positive Korrelationen können das Resultat antigener Ähnlichkeit der Parasiten (die die Ausnutzung der Immunmodulation durch den anderen Parasiten erlaubt) oder der Tatsache, dass die Parasiten mit Wirten ähnlicher ökologischer Spezialisierung assoziiert sind, sein. Negative Beziehungen können auf Konkurrenz, die möglicherweise in Zusammenhang mit der phylogenetischen Verwandtschaft der Parasiten oder mit der Ähnlichkeit im Ressourcenbedarf steht, beruhen. Zwischen den Wirtsarten unterschieden sich Richtung und Ausprägung der Parasiteninteraktionen nicht, was darauf hindeutet, dass intrinsische Merkmale der Wirtsarten die Beziehungen der Parasiten untereinander nicht beeinflussen, was nicht im Einklang mit der Hypothese einer Wirtsspezifität steht.

Die Fortpflanzungsaktivität der Copepoden (gemessen als Anteil der Weibchen, die Eiergelege tragen) unterschied sich zwischen den Wirtsarten weder im Feld (**Kapitel 5**) noch im Labor (**Kapitel 4**). Dies deutet auf keine Wirtsspezifität der Fortpflanzungsaktivität von Copepoden hin.

SCHLUSSFOLGERUNG

Diese Arbeit unterstützt die Hypothese, dass Parasiten zur Wirtsdivergenz beitragen, diese aber nicht initiieren. Zum einen sind Parasiten nicht zufällig auf mindestens drei Ebenen - Kiemen-Mikrohabitat, Wirtsarten, Wirtslinien - verteilt, was auf eine Wirtsspezialisierung und eine Möglichkeit zur heterogenen, parasitenvermittelten Selektion hindeutet. Zum anderen waren die Artenunterschiede bei der Infektion zeitlich konsistent, was den Voraussetzungen einer parasitenvermittelten Speziation entspricht. Darüber hinaus zeigen Wirtsarten, deren ökologische Speziation gerade begonnen hat, zunehmende Unterschiede in den Parasitengemeinschaften, was darauf hindeutet, dass die Differenzierung der Infektionen eher ein Nebenprodukt der Divergenz als ihr Ausgangspunkt ist.


Grazie
Dank u
Merci
Thank you
Danke

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Declaration of consent

on the basis of Article 18 of the PromR Phil.-nat. 19 (University of Bern)

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